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CENTRAL INHIBITION IN THE SYMPATHETIC NERVOUS SYSTEM

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by

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Ph.D. Physiology

It is known that CNS mechanisms for inhibition of sympathetic activity exist, but the underlying circuitry and cellular mechanisms are largely unknown. As an attempt to clarify these points, the inhibitory effect, on single antidromically identified sympathetic preganglionic neurons, of electrical stimulation of high threshold somatic and visceral afferents, descending fibres in the lateral funiculus of the spinal cord and depressor area of the medulla oblongata, near the obex, have been examined. With extracellular recording, inhibition was seen as a depression of spontaneous or glutamate-evoked firing and of antidromic soma invasion, unrelated to a previous excitation. All the inputs studied could inhibit glutamate-evoked activity and, less frequently, injury discharge and antidromic soma invasion. These results suggest the possibility that the inhibition of sympathetic activity is, at least in part, by a postsynaptic mechanism operating on the sympathetic preganglionic neuron membrane. This inhibition was strychnine-resistant and picrotoxin sensitive.

INHIBITION CENTRALE AU NIVEAU DU SYSTEME NERVEUX SYMPATHIQUE

par

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Ph.D. Physiologie

On connait l'existence au niveau du SNC, de mécanismes inhibiteurs de l'activité sympathique mais on ignore en général leur organisation et leurs mécanismes cellulaires. Dans le but d'élucider ces points, on examina l'action inhibitrice sur des neurones sympathiques préganglionaires identifiés antidromiquement, de stimulation électrique de voies afférentes somatiques et viscérales à seuil éléve des fibres descendantes du funiculus latéral de la moelle épinière et de la région dépressive du bulbe près de l'obex. Lors de l'enregistrement extracellulaire l'inhibition se manifesta par une diminution de décharges spontanées ou produites par le glutamate et par stimulation antidromique. Tous les inputs etudiés pouvaient inhibiter l'activité produite par le glutamate, et moins fréquemment, les décharges résultant de "injury" et de la stimulation antidromique. Ces résultats suggerent que l'inhibition de l'activité sympathique est la résultat d'un mécanisme postsynaptique agissant sur la membrane du neurone sympathique préganglionaire. Cette inhibition s'avéra strychnine-résistante et picrotoxine-sensible.

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ABBREVIATIONS

SPN - sympathetic preganglionic neuron

FRA - flexor reflex afferent

DRP - dorsal root potential

CSN - carotid sinus nerve

NTS - nucleus tractus solitarius

PED - post-excitatory depression

CNS - central nervous system

EPSP - excitatory postsynaptic potential

IPSP - inhibitory postsynaptic potential

C-T - conditioning-testing

AHP - afterhyperpolarization

WR - white ramus

PSH - post-stimulus histogram

T - threshold

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1. INTRODUCTION

A. General Considerations Concerning Inhibition in the Sympathetic Nervous System.

Inhibitory mechanisms seem to be operating at all levels of the sympathetic nervous system. Available experimental evidence (Beck and Brody, 1961) suggests that some inhibitory mechanisms act directly on the effectors (peripheral inhibition) while others operate within the central nervous system (central inhibition). Still other inhibitory mechanisms seem to be present in the ganglia (Libet, 1967).

Peripheral inhibitory mechanisms would induce relaxation of smooth muscle (vascular, for instance) regardless of the presence of a CNS-generated "neurogenic" constrictor tone. For the vascular smooth muscle of skeletal muscle, from which most of the available information has been obtained, at least three types of probable peripheral inhibitory mechanisms have been distinguished on the basis of their pharmacology and, by inference, of the transmitter involved: a cholinergic muscarinic (Uvnäs, 1954), a beta-adrenergic (Viveros et al., 1968) and a histaminergic (Beck, 1965). Neurophysiological data, proving the real inhibitory nature of these mechanisms, are still, however, lacking (Somlyo and Somlyo, 1970, see p. 272, 275 and 286).

Experimental data suggest that inhibitory mechanisms might also be operating at the sympathetic ganglia (Libet, 1967). Transmission through the ganglia could be depressed by the inhibition, although direct evidence that this really happens is still lacking. This

inhibitory mechanism appears to be mediated by a catecholamine and associated with IPSP generation in the ganglion cells (Libet and Tosaka, 1970).

Finally, there seem to be inhibitory mechanisms operating on the central nervous components of the sympathetic nervous system. The existence of this central inhibition has been originally inferred from observations of effector behaviour, for instance, from observations that stimulation of peripheral afferents or of CNS structures could evoke blood pressure falls and a depression of tonic activity of smooth muscle or glands. These effects are absent if the basal neurogenic "tone" of the effectors is eliminated (Folkow, 1956). However, only for high threshold limb afferents has direct evidence for a central inhibitory effect been obtained by recording from single sympathetic preganglionic neurons (SPNs) (Wyszogrodski, 1970; Jänig and Schmidt, 1970). Other peripheral afferents with postulated, but not yet proven, inhibitory action on sympathetic neurons are arterial baroreceptor, high threshold visceral, and some special sense, like optic and vestibular afferents. Structures at various levels of the CNS evoke, when stimulated, sympathetic effector behaviour which has been attributed to a hypothetical central inhibition. A list includes sites in the medulla and pons, mesencephalon, cerebellum, hypothalamus, limbic system, and motor cortex (Uvnäs, 1960). As mentioned above, convincing neurophysiological data proving the inhibitory nature of certain reflex and centrally evoked effector behaviour exist only for the high threshold somatic

afferents. For all the other inputs, unequivocal demonstration that they can evoke true inhibition is still lacking. Consequently, the central organization of all these postulated inhibitory pathways and the underlying neurophysiological processes are unknown. These are the main problems with which the present work is concerned and will be outlined more precisely in section C. In the following section, existing data are reviewed relevant to the topic of central inhibition of the sympathetic nervous system.

B. Survey of Earlier Studies Related to Inhibition in the Sympathetic Nervous System.

(i) Spinal Somatic Afferents

The earliest evidence, suggesting an inhibitory effect of these afferents, was the observation that repetitive electrical stimulation of limb nerves or of spinal dorsal roots could evoke, under some conditions, a fall of arterial pressure due to a decrease in peripheral resistance (cf. Johansson, 1962, for an extensive review of the relevant literature). This reflex decrease in peripheral resistance was attributed to a central depression (i.e. inhibition) of vasoconstrictor tone (loc.cit.). It would seem however that neither pressure nor flow measurements could supply conclusive proof for the central inhibitory nature of this phenomenon. For instance, a weak, asynchronous excitation of vasoconstrictor neurons, followed by a relatively powerful and long-lasting post-activity depression, which would turn off their background firing, could probably evoke effects similar to those described in a slow system like the sympathetic

neuro-effector system. Also, a desynchronization of the background activities of the vasoconstrictor neurons could produce the same effect, by reducing the synaptic efficacy of their tonic activity at the ganglion and at the neuro-effector junction. Other sympathetic effectors, besides vascular smooth muscle also appear to be depressed (for instance, the nictitating membrane - Acheson et al., 1936).

More recently, electrophysiological data have shown that somatic afferent stimulation can depress background and reflex discharges in pre- and post-ganglionic neurons. Most of the data were obtained in multifibre preparations (Sell et al., 1958; Khayutin, 1964; Beacham and Perl, 1964; Fedina et al., 1966; Alanís and Defilló, 1968; Koizumi et al., 1968; Iwamura et al., 1969; Coote and Perez-Gonzalez, 1970). With this type of preparation it is difficult to establish with certainty whether the depression is the result of a true inhibitory process or of a post-activation depression or of a de-synchronization of firing. However, recordings from single axons (Jänig and Schmidt, 1970) or cell bodies (Wyszogrodski, 1970) of sympathetic preganglionic neurons have shown that the depression of background activity of single units can be evoked by somatic afferent stimulation and this occurs without prior excitation, thus establishing that this phenomenon is likely to be a true inhibition.

Some studies have utilized the blood pressure fall (Laporte and Montastruc, 1957; Skoglund, 1960; Laporte et al., 1960), others electrophysiological methods (Koizumi et al., 1968; Coote and Perez-Gonzalez, 1970), to determine the type of afferents evoking the

inhibitory reflex. There seems to be general agreement that group III afferents from skin or muscle can inhibit sympathetic activity. Group I afferents from muscle and group IV appear to have no inhibitory effect on the sympathetic, while group I cutaneous and group II muscle afferents' involvement in the inhibitory reflex is controversial. Thus the inhibitory fibres are within the group of afferents usually called "flexor reflex afferents" (FRAs) because the flexor reflex is one of the best known manifestations of their various central actions.

Concerning the central circuitry and the mechanism of this inhibitory reflex, the available data are inconclusive. Johansson (1962) has shown that cats with bilateral lesions of the ventromedial part of the medulla oblongata at the level of the pyramidal decussation or of the ventrolateral funiculi of the cord at C_1 do not show blood pressure falls in response to stimulation of somatic afferents. These findings are in general agreement with those of Brooks (1933), Fernandez de Molina and Perl, (1965), Koizumi et al., (1968), Kučera (1969) and Coote and Perez-Gonzalez (1970), who did not observe any depressor responses by stimulating somatic afferents in chronic and acute spinal cats. On the basis of such evidence, the inference was made that the circuitry of this inhibitory reflex included a supraspinal relay. However, alternative explanations could account for these observations. In particular, a reflex that evokes a fall in blood pressure, by a mechanism of inhibition of neurogenic tone, may be ineffectual in the spinal animal, simply because neurogenic tone is absent. On the basis of the data presented by the above authors it cannot be established

whether spontaneous neurogenic tone was present in their spinal animals. Some support to the idea of a supraspinal relay came from later electrophysiological data. Iwamura et al., (1969) found that the silent period, which follows the reflex discharge evoked in whole preganglionic nerves by somatic afferent stimulation, was abolished in cats with a lesion of the medulla oblongata (ventral reticular formation at the level of the inferior olive). This area corresponds to the so-called "depressor" area of Chai and Wang (1962). Koizumi et al., (1968) and Coote and Perez-Gonzalez (1970) found similarly that an excitatory sympathetic reflex, evoked by somatic afferents, was depressed after a conditioning stimulus (which by itself evoked a reflex) to the same afferents, for a much shorter duration in the acute or chronic spinal than in the intact animal. However, the interpretation of these recovery curves of an excitatory reflex is not simple: the observed depression of the test reflex could be due to subnormality, besides inhibition, and in any case it would also be dependent on the amount of excitation which is being evoked. Since the same authors in fact report that the size of the "early" reflex* was increased in the spinal animal as compared

* Stimulation of high threshold somatic afferents gives origin, in the cat with intact neuraxis, to short-latency (early) and long-latency (late) reflex excitation of SPNs (Sato et al., 1965; Coote and Downman, 1966; Sato and Schmidt, 1971). The early reflex is largely segmental, the late intersegmental. The late reflex, but not the early, is abolished in the spinal animals. (Sato and Schmidt, 1971). On this basis the early and late reflexes are attributed to the operation of a spinal and spinal-bulbo-spinal circuit respectively.

with the neuraxially intact, this factor by itself could probably account for the shortening of the time course of reflex recovery in the spinal animal. On the other hand, Okada et al., (1960) found that tetani to the sciatic nerve could inhibit the spontaneous activity of the long ciliary nerve in the acute spinal cat. However, these responses were seen occasionally. It must be pointed out, however, that spontaneous activity in the long ciliary nerve was much reduced after spinal section. This factor by itself would contribute to a reduction in the likelihood of observing inhibition.

Concerning the mechanism of the inhibition, no data areavailable in the literature. For instance, it is not known whether the inhibition is associated with a change in excitability of the SPN or not. Sato and Schmidt (1966) suggested, without experimental basis however, that the inhibition could be analogous in mechanism to the presynaptic inhibition caused by the FRAs on themselves and on other afferent systems, i.e. due to a depolarization of afferent terminals. From a study of the effects of strychnine and picrotoxin on the recovery of an excitatory somato-sympathetic reflex, Franz (1966) also suggested that the inhibition could be of the presynaptic type. However, this hypothesis has not yet been submitted to any kind of experimental test.

(ii) Spinal Visceral Afferents

As for the somatic afferents, one line of evidence suggesting the possibility of a central inhibitory effect of these afferents on sympathetic neurons was the observation, under certain experimental

conditions, of blood pressure falls resulting from repetitive electrical stimulation of splanchnic nerve afferents in experimental animals (Burton-Opitz, 1916, 1917). Blood pressure falls were also obtained by stimulation of afferents in the renal and mesenteric nerves and were attributed to a decrease in total peripheral resistance due to central inhibition of tonically active vasoconstrictor neurons (Johansson and Langston, 1964; Khayutin, 1966; Ueda et al., 1967; Aars and Akre, 1970). A galvanic skin response, evoked by stimulation of limb nerves, could also be depressed by stimulation of visceral afferents in the splanchnic nerve (Kumazawa and Naotsuka, 1970).

Electrophysiological studies in animals with an intact neuraxis have shown that electrical activity in sympathetic nerves can be reduced by visceral nerve stimulation (Fedina et al., 1966; Ueda et al., 1967; Aars and Akre, 1970). This finding suggests that these afferents may cause inhibition. However, this depression of tonic activity has been seen by some investigators only as a sequel to an early reflex excitation evoked by the same afferents (Iriuchijima, 1959; Fedina et al., 1966). After a single shock to the mesenteric nerves, the period of depression lasted 400 msec (Fedina et al., 1966). Thus, like for the somatic afferents, there is the problem of whether the depression of background activity is due to inhibition or to other processes. No single unit study was available, prior to the present one.

The visceral afferent fibres, evoking the reflex depression, appear to be the same that cause reflex excitation: they are myelinated fibres with conduction velocities between 15 and 35 m/sec (Franz et al.,

1966; Kumazawa and Naotsuka, 1970). Worth mentioning is that these afferents have conduction velocities similar to those of the already mentioned somatic afferents which also evoke sympathetic inhibition.

Ablation of the medulla does not influence the early reflex sympathetic excitation, evoked by these afferents, but eliminates the late depression (Fedina et al., 1966). On the assumption that the late depression was due to inhibition, this observation would suggest that the inhibitory pathway is not complete, or not operative, in a spinal cord isolated from higher centres. In contrast, Franz et al., (1966) recording from white rami in the acute spinal animal, found that a viscerosympathetic reflex discharge was depressed for up to 400 msec by a conditioning volley in the same visceral nerve. Similarly, there was a depression of a somato-sympathetic reflex discharge by a conditioning shock to a visceral nerve. This depression was attributed by the authors to inhibition, rather than to post-activity depression, because of the relatively small size of the conditioning volley (which, however, never failed to evoke a discharge. If it failed, the depression was also absent). If these phenomena, as the authors suggest, are inhibitory, then one must conclude that a spinal pathway exists for this type of inhibition. The further observation that i.v. picrotoxin can reduce the duration of the reflex depression (Franz, 1966), together with the reported facts that picrotoxin depresses presynaptic inhibition (Eccles et al., 1963) and that visceral afferents depress polysynaptic motoneuron reflexes from muscle afferents, without altering motoneuron excitability (Duda,

1964), led Franz et al., (1966) to suggest that viscero-sympathetic inhibition was probably analogous in mechanism to presynaptic inhibition. Work by Hancock et al., (1970) has shown that DRPs can be evoked by splanchnic afferents. In summary, data concerning this hypothetical inhibitory pathway arescarce. Unequivocal demonstration that the various depressant effects reported were due to inhibition is still lacking, due to the lack of single unit data. Whether the hypothetical inhibitory synapses are in the spinal cord and whether the pathway is complete within the cord are also still open questions. Neither is it known whether excitability changes of the SPN are associated with this hypothetical inhibition. Similarities exist between the depression of sympathetic activity evoked by somatic and visceral afferents: the depression is of long duration, is sensitive to picrotoxin and is evoked by afferents belonging to the same fibre size range.

(iii) Carotid Sinus Afferents

The carotid sinus nerve (CSN), a branch of the IXth cranial nerve, contains the primary afferent fibres from baroreceptors of the carotid sinus wall and chemoreceptors of the carotid body. This nerve has attracted a great deal of interest because of its role in the homeostatic regulation of arterial pressure (Heymans and Neil,

1958: Kezdi, 1967). Excitation* of the carotid sinus baroreceptors causes a fall of arterial pressure, due partly to bradycardia and partly to peripheral vasodilatation (Folkow et al., 1950; Frumin et al., 1953; Lindgren and Uvnäs, 1954). The vasodilatation appears to be due to a central depression of vasoconstrictor tone (Folkow, 1956; Lindgren and Uvnäs, 1954).

Electrophysiological studies have shown that carotid sinus baroreceptor excitation causes a marked depression of the background discharge of whole sympathetic nerves (Pitts et al., 1941; Gernandt et al., 1946; Gernandt and Zotterman, 1946; Cannon et al., 1954; Koizumi and Suda, 1963: Downing and Siegel, 1963; Weidinger and Leschhorn, 1964; Koizumi et al., 1968; Coote and Downman, 1969; Coote et al., 1969). This depression of sympathetic activity has been considered the cause of the blood pressure fall already described. An inverse relationship exists between carotid sinus pressure and the amount of activity in a whole sympathetic nerve (Kezdi and Geller, 1968). This reflex is tonically active as shown by the fact that denervation of baroreceptors causes a) a blood pressure rise and b) a loss of "cardiac" modulation

* Baroreceptor excitation can be done with open or closed loop methods. Open loop methods are electrical stimulation of the central cut end of the CSN or the distention with pressure pulses of the isolated carotid sinus (Moissejeff, 1927). A closed loop method is the injection of a "pressor" drug into the circulation, the resulting blood pressure rise causing the excitation of the baroreceptors. Electrical stimulation of the CSN is not a selective way of exciting baroreceptor fibres because of the presence of chemoreceptor afferents of similar size, and hence threshold, in the CSN.

of sympathetic discharges (Adrian et al., 1932; Bronk et al., 1936; Downing and Siegel, 1963; Green and Heffron, 1968; Gabriel and Seller, 1969; Cohen and Gootman, 1970). Reflexly evoked discharges of sympathetic nerves can also be depressed by baroreceptor excitation (Coote and Downman, 1969; Coote and Perez-Gonzalez, 1970; Kirchner et al., 1971; Koizumi et al., 1971). Studies of single sympathetic units have not yet been done.

The carotid sinus baroreceptor afferents are of two types, one group consisting of myelinated fibres of 2 to 8 microns (A-fibres) in diameter, with a second group consisting of small non-myelinated fibres of less than 2 microns in diameter (DeCastro, 1926, 1951; Douglas and Ritchie, 1956; Douglas and Schaumann, 1956; Eyzaguirre and Uchizono, 1961; Fidone and Sato, 1969). Large decreases in arterial pressure (greater than 25 mm Hg) are only obtained if electrical stimulation is intense enough to excite these small unmyelinated fibres (Douglas and Ritchie, 1956).

Concerning the central circuitry of this presumably inhibitory reflex, a suggestion that a relay in this reflex pathway might be in the dorsal part of the medulla oblongata came from experiments showing the similarity of cardiovascular responses to electrical stimulation of this area and of the carotid sinus nerve (Ranson and Billingsley, 1916; Scott and Roberts, 1923; Scott, 1925; Lindgren and Uvnäs, 1953; Lindgren, 1955; Hellner and Baumgarten, 1961; Calaresu and Pearce, 1965). Histological controls showed that the stimulated area causing these effects was the nucleus tractus

solitarius (NTS) (Hellner and Baumgarten, 1961; Calaresu and Pearce, 1965). Degeneration studies showed that the main site of termination of CSN afferent fibres is the middle third of the NTS, at the level of and just rostral to the obex (Kerr, 1962; Cottle, 1964; Rhoton et al., 1966). Accordingly, a lesion in the area of the NTS may abolish the carotid sinus reflex (Yi, 1938; Lindgren and Uvnäs, 1954; Oberholzer, 1955; Löfving, 1961a). More recently, electrophysiological data have become available. Monosynaptic field and unit potentials have been evoked in the NTS by CSN stimulation. Primary afferent fibres of the CSN can be excited antidromically by NTS stimulation (Humphrey, 1967; Sampson and Biscoe, 1968; Seller and Illert, 1969; Miura and Reis, 1969). All these observations, taken together, strongly suggest that the first synapse of the inhibitory pathway is in the NTS. The latencies of the NTS responses to CSN stimulation were between 0.7 and 13 msec (Humphrey, 1967; Sampson and Biscoe, 1968; Miura and Reis, 1969; Seller and Illert, 1969). Another site in the medulla where monosynaptic responses to CSN stimulation have been recorded (Miura and Reis, 1969) and from which primary CSN afferent fibres can be excited antidromically (Crill and Reis, 1968) is in the medial reticular formation (Nucleus paramedianus reticularis - Taber, 1961), in the general area designated as the "depressor" area by Alexander (1946). This is then another possible site of the first synapse of the carotid sinus inhibitory pathway. Polysynaptic field and unit responses to CSN stimulation with latencies of 3-8 msec (Humphrey, 1967; Sampson and Biscoe, 1968), and as high as 80 msec (Miura

and Reis, 1969) have been recorded both in the medial and lateral regions of the medullary reticular formation. These findings suggest that the second and higher order relays of the CSN inhibitory pathway may be located in these areas. However, no direct projections were found by degeneration studies, in the cat, from the NTS to the medial reticular formation (Morest, 1967) but polysynaptic field potentials evoked by NTS stimulation have been seen by Humphrey (1967). While the message from the CSN gets to the first synapse in a few msec (Humphrey,1967; Sampson and Biscoe, 1968; Miura and Reis, 1969; Gabriel and Seller, 1970), the latency for depression of discharge in sympathetic nerves is much longer. Richter, Keck and Seller (1970) found an average latency of 181 msec for the complete suppression of background activity in the abdominal sympathetic chain by tetanic stimulation of the CSN. Thus, there is a very long central transmission time to be accounted for.

A question which has not yet been answered is where are the inhibitory synapses in this supposedly inhibitory reflex pathway, in other words at what level of the central nervous system does inhibition of sympathetic activity by baroreceptor afferents occur? This seems an important thing to establish before any attempt to discover the mechanism of the inhibition can be made. This question has been approached in the following, rather indirect, way. As mentioned earlier (footnote on p. 6), somatic afferents evoke early and late reflex discharges in sympathetic nerves which are attributed to the operation of a spinal and spino-bulbo-spinal circuit, respectively.

Koizumi et al., (1971) found that the late, but not the early, reflex could be depressed by baroreceptor stimulation (distention of an isolated carotid sinus with all other baroreceptor nerves cut): this observation led to the interesting inference that the inhibitory synapses were not, probably, in the spinal cord, but were in the medulla. However, nearly simultaneously Kirchner et al., (1971) published that baroreceptor stimulation (by i.v. injection of a pressor drug) did depress both early and late somato-sympathetic reflexes, although the late was depressed to a greater extent than the early. Clearly, this approach seems too indirect to expect that it would give a conclusive answer to the question as to the site of the inhibitory synapses, in particular since the notions concerning the circuitry of the excitatory somato-sympathetic reflexes are far from being definite. Thus the problem of the mechanism of baroreceptor inhibition is still unsettled. The only possibly relevant work available is that of Biscoe and Sampson (1970) on the effect of CSN stimulation on phrenic motoneurons. Hyperpolarizing potentials were observed with latencies of 5 to 10 msec. No changes in membrane conductance were detected suggesting that the hyperpolarization of phrenic motoneurons was due to a disfacilitation. The disfacilitation was blocked by strychnine. However, it remains to be established whether the inhibition of SPNs by the CSN has similar characteristics.

(iv) Aortic Nerve Afferents

The aortic nerve, a branch of the Xth cranial nerve, contains the

primary afferents from the baroreceptor areas in the aortic arch, the origin of the subclavian artery (Koester and Tschermak, 1903, see Heymans and Neil, 1958, p. 26) and the common carotid artery (Boss and Green, 1956). Excitation of these afferents evokes a blood pressure fall due to bradycardia and peripheral vasodilatation (Cyon and Ludwig, 1866): these, in turn, appear to be due, at least in part, to central inhibition of cardio-accelerator and vasoconstrictor tone (Folkow, 1956).

Increasing the pressure in the aortic arch or electrical stimulation of the aortic nerve evokes a depression of the background discharge of sympathetic nerves which in turn has been considered the cause of the blood pressure falls (Bronk et al., 1936; Frumin et al., 1953; Okada, 1964). The aortic nerve afferents, like the carotid sinus afferents, probably exert a tonic inhibitory action on the sympathetic neurons, because they fire tonically at normal blood pressure values (Heymans and Neil, 1958, Fig. 12) and because their elimination, in preparations without other functioning baroreceptors, causes an increase in blood pressure and changes in the discharge patterns of sympathetic nerves, i.e. loss of cardiac modulation (Bronk et al., 1936).

The baroreceptor fibres of the aortic nerve closely resemble in their properties those of the carotid sinus nerve baroreceptors. Both A and C fibres occur in the aortic nerves of cats and rabbits (Douglas et al., 1956; Agostoni et al., 1957). The conduction rate of the A fibres in the aortic nerve is 33±11 m/sec (Paintal, 1953). By electrical stimulation of the aortic nerve, low intensities evoke depressor

responses, as a result of low threshold baroreceptor A-fibre activation; higher intensities evoke pressor effects, as a result of higher threshold chemoreceptor A-fibre activation; still higher intensities evoke depressor responses again, as a result of non-medullated fibre activation (Douglas and Schaumann, 1956). It is not known, however, whether the small unmyelinated fibres are connected to baroreceptors or to other types of receptors.

Not much information is available concerning the central circuitry of this hypothetical sympatho-inhibitory reflex. Lesions of limited areas of the medulla in the region of the obex abolished both the aortic and carotid sinus depressor reflexes. Degeneration studies have shown that the sites of termination of the primary afferents of the aortic nerve are similar to those for the carotid sinus afferents, i.e. in and around the nucleus tractus solitarius (Kerr, 1962; Cottle, 1964; Rhoton et al., 1966), particularly in the area adjacent and rostral to the obex. It is possible that both the aortic and CSN afferents utilize the same circuitry in the central nervous system. Additional degenerating fibres, after section of the aortic nerve, have been found in the descending tract of the Vth nerve, in the nucleus cuneatus medialis and in the reticular nuclei of the medulla (Kerr, 1962; Rhoton et al., 1966). Studies on the distribution of evoked field and unit potentials elicited by electrical stimulation of the aortic nerve have provided data in agreement with much of the data from degeneration studies (Anderson and Berry, 1956; Crill and Reis, 1968; Gabriel and Seller, 1970). Evoked responses were found in the

NTS, in nuclei of the medial reticular formation corresponding to the so-called "depressor" area (Alexander, 1946) and in the cuneate nucleus. Stimulating the same sites in the medulla, antidromic action potentials could be recorded in fibres of the aortic nerve, with a conduction velocity from 16 to 40 m/sec (Crill and Reis, 1968).

(v) Cardiopulmonary Afferent Fibres in the Vagus Nerve other than Aortic Baroreceptors

In addition to baroreceptor afferents from the aortic arch, which are not always segregated in the aortic nerve, the cervical vagus contains a number of other afferents which might have an inhibitory effect on the sympathetic and can, therefore, be responsible for the blood pressure falls and relaxation of the nictitating membrane (Molnár et al., 1969) evoked by maximal electrical stimulation of this nerve. Some of these afferents originate from baroreceptors in the pulmonary artery which are similar in properties to the better known sinus and aortic baroreceptors. Their activation causes bradycardia, hypotension and apnoea (see Bevan, 1967, for review). Another group of afferents are those from receptors activated by stretch of, and injection of certain drugs in, the right and left heart (see Aviado and Schmidt, 1955 for review), which reflexly evoke arterial hypotension and a fall in peripheral resistance. Finally, there are some data suggesting that pulmonary stretch receptors which evoke the Hering-Breuer reflexes have a depressant effect on vascular resistance, and that this effect also is mediated by the vagus (Daly et al., 1967; Glick et al., 1969).

Apart from the data obtained by stimulating the receptors, observations have been reported that section or block of the cervical vagus, in animals with sino-aortic denervation, produces an increase in blood pressure: this would suggest the existence of a tonic, non-aortic baroreceptor, inhibitory influence of vagal afferents (Guazzi et al., 1962a, b, c; Öberg and White, 1970). The fibre types responsible for this array of reflex effects are not known. Nor is there any neurophysiological evidence concerning the effects of all these receptors on sympathetic neurons. Thus, whether all the above phenomena are due to nervous inhibition or to other mechanisms is not known. Concerning the central nervous system circuitry mediating the effects described, the only possible relevant information is that lesions in the so-called "depressor" area of the medulla (Johansson, 1962; Wang and Chai, 1967) abolish the depressor responses to cervical vagus afferent stimulation.

(vi) Afferents in other Cranial Nerves

Some responses of sympathetic effectors, resulting from stimulation of the I (optic), VIII (vestibular) and XII (hypoglossal) nerves, could be due to central inhibition of the sympathetic. Molnár et al., (1969) reported that strong illumination of the eye can evoke a relaxation of the innervated nictitating membrane in cats. Earlier, Nisida et al., (1960) had observed that sustained photic stimulation can suppress the background discharge of the long ciliary nerves. This suppression appears in the absence of any prior excitation, thus

suggesting a true inhibitory phenomenon. No further studies of the properties of this inhibition have been made.

A possible inhibitory effect of the vestibular nerve on the sympathetic was first suggested by the observation of Spiegel and Démétriades (1922). They reported that stimulation of the VIII nerve or of the labyrinth in rabbits could evoke blood pressure falls. This observation was later confirmed by several authors (Spiegel and Démétriades, 1924; Pupilli, 1926; Mark and Seiferth, 1934; Megirian and Manning, 1967). More recently, an investigation of the effects of vestibular nerve stimulation on the tonic electrical activity of bundles of sympathetic post-ganglionic axons in the renal nerve of the cat has been made by Uchino et al., (1970). Electrical stimulation of the vestibular nerve with short trains of pulses, evoked a suppression of tonic activity with a latency of 80 msec and lasting approximately 1 second. This effect appeared at a stimulus intensity twice the threshold (T) for the nerve and was maximal at 5-6 times T. Stimuli of the same parameters also evoked the already described blood pressure fall. Finally, Whitwam et al., (1969) showed that electrical stimulation of group III afferents in the hypoglossal nerve evoked a mixed response in the renal nerve, i.e. excitation followed by a silent period. Whether the silent period was due to a post-excitatory depression or to an inhibition, was not clarified by these authors.

(vii) Medulla Oblongata

Electrical stimulation of sites in the medulla oblongata has been shown to evoke blood pressure falls (Ranson and Billingsley, 1916; Lim et al., 1938; Monnier, 1939; Wang and Ranson, 1939; Frumin et al., 1953; Lindgren and Uvnäs, 1953, 1954; Hellner and Baumgarten, 1961; Chai and Wang, 1962; Calaresu and Pearce, 1965) which have been attributed to central inhibition of vasoconstrictor tone (Frumin et al., 1953; Lindgren and Uvnäs, 1954). All these sites are designated as the depressor area (Ranson and Billingsley, 1916; Alexander, 1946). This depressor area is rather large and includes a number of structures such as the nucleus tractus solitarius, the medial reticular formation and the cuneate nucleus (Wang and Ranson, 1939; Alexander, 1946; Bach, 1952). This is an area from which inhibition of somatic reflexes can also be obtained (Magoun and Rhines, 1946; Alderson and Downman, 1966). The sympatho-inhibitory effects obtained by stimulation of this region could be due to activation of an inhibitory system originating in the medulla itself, or of inhibitory pathways originating elsewhere and coursing through the medulla (for instance originating from baroreceptor afferents, or descending from higher central nervous system structures). More recently, it has been shown that electrical stimulation of the medulla can suppress the background electrical activity of renal nerves (Scherrer, 1966) or of splanchnic nerves (Kahn and Mills, 1967; Gootman and Cohen, 1971). Scherrer's depressor effects were obtained from two well defined areas, one corresponding to the tractus solitarius and its nucleus (latency for the effect:

200-500 msec), the other corresponding to the general region of the medial longitudinal fasciculus and the medial tecto-spinal tract, at the level of the rostral part of the hypoglossal nucleus (latency for the effect: 100-150 msec). Gootman and Cohen's effects were obtained from points in the ventromedial reticular formation, just rostral to the obex, and had a latency of 30 msec and a duration of 20-30 msec. There is some evidence, not entirely convincing, however, because of the lack of adequate controls, that this hypothetical medullary inhibitory system exerts a tonic influence on sympathetic neurons: a spinal transection at C-1, secondary to a transection at the obex, resulted in an increase in background discharge of the inferior cardiac nerve, suggesting a release from a tonic inhibition (Alexander, 1946). Reflexly evoked sympathetic discharges can also be suppressed by medullary stimulation. Intercostal to renal nerve (Coote and Downman, 1969) and intercostal to white ramus (Coote et al., 1969) reflexes could be suppressed by stimulation of sites within the ventromedial medullary reticular formation. A galvanic skin response and a vasoconstrictor reflex, evoked by somatic afferent stimulation, could also be inhibited by stimulation of the ventromedial medullary reticular formation (Wang and Brown, 1956; Prout et al., 1964).

(viii) Supramedullary Structures

The data available are mostly observations of blood pressure falls upon stimulation of various structures. A study of blood pressure

alone is not a reliable guide to understanding neurophysiological processes underlying these responses. In many instances, vagal and respiratory effects, and skeletal muscle movement, were not eliminated, so that one cannot say whether the blood pressure effects observed were the primary consequence of the stimulus and mediated via the sympathetic system, or were they effects secondary to other changes evoked by stimulation in other systems. Few, more controlled, experiments have been made (e.g. Löfving, 1961b). No neurophysiological data are available. Thus, it must be understood that the large amount of data reviewed below are only suggestive of the existence of inhibitory effects.

Hypotensive responses have been recorded to stimulation of various mesencephalic regions. These areas include the nucleus of the posterior commissura, the substantia grisea centralis, the tegmentum, and the anterior portion of the tectum (Kabat et al., 1935). These responses could be due to inhibition of tonic activity of vasoconstrictor neurons. They could be attributed to stimulation of neural pathways originating in the site stimulated, or simply passing through it.

Hypotensive responses can be elicited by electrical stimulation of points within the hypothalamus (Karplus, 1937; Hess, 1938; Ranson and Magoun, 1939; Pitts et al., 1941; Thompson and Bach, 1950). Experiments by Folkow et al., (1959) of stimulation of a discrete depressor area just caudal to the anterior commissure, showed that the hypotensive response was due to a suppression of vasoconstrictor tone (also Uvnäs, 1960). It is of interest that the anterior hypothalamus

is designated as a heat loss centre and controls particularly skin vascular resistance (Strŏm, 1950). Of interest, is the finding of direct connections (i.e. axonal) between hypothalamus and intermediomedial regions of the thoracic spinal cord (Smith, 1965).

Electrical stimulation of motor and premotor cortex has sometimes produced hypotensive effects suggesting suppression of vasoconstrictor tone (Hoff and Green, 1936; Green and Hoff, 1937; Hsu et al., 1942; Morin and Zwirn, 1953). Marked hypotensive responses can be obtained from stimulation of the more anterior parts of the orbital cortex, the anterior parts of the temporal lobe (including the amygdala), the insula and the anterior cingulate gyrus (Smith, 1945; Speakman and Babkin, 1949; Kaada et al., 1949; Kaada, 1951; Hess et al., 1951; Wall and Davis, 1951; Anand and Dua, 1956; Lŏfving, 1961b).

The sympatho-inhibitory fibres of the cingulate depressor area appear to pass via the sympatho-inhibitory area in the anterior hypothalamus. An electrolytic lesion of this structure eliminated fairly selectively the cortically induced depressor effects (Lŏfving, 1961b). From some cortical regions, however, vasomotor fibres run together with the pyramidal tracts and continue to function even after destruction of the entire hypothalamus (Wall and Davis, 1951). There thus seem to be both extrapyramidal and pyramidal cortico-fugal vasomotor fibres (Hunsicker and Spiegel, 1934; Spiegel and Hunsicker, 1936; Green and Hartzell, 1938). Electrolytic lesion of the medullary depressor area abolished both cortically-induced depressor effects

(Löfving, 1961b). These findings suggest that all those sympatho-inhibitory mechanisms emanating from cortical structures exert their cardiovascular effects via the medial part of the medullary reticular formation, which constitutes the depressor area of the vasomotor centre (Löfving, 1961b). In summary then, little is known about the anatomical pathways mediating these autonomic effects, although the physiological evidence suggests that cortical influences relay in hypothalamic and medullary nuclei, in addition to using more direct pathways through the pyramidal tract.

C. Aim of the Present Study.

In the preceding section, a number of experimental observations have been reviewed, which might have, as their common underlying mechanism, a central inhibition of the sympathetic nervous system. However, convincing proof that these phenomena are due to nervous inhibition is still lacking, due to the lack of single unit studies. The only exception, as mentioned earlier, is the depressant effect of high threshold somatic afferents on sympathetic activity, the true inhibitory nature of which has been demonstrated by Wyszogrodski (1970) and Jänig and Schmidt (1970).

Thus, one of the aims of the present work was to establish for an additional spinal input (visceral afferents in the splanchnic nerve) and for supraspinal inputs (afferents in the cervical vagus nerve, sites in the medulla and descending tracts in the cervical cord), whether or not they exert a true inhibitory action on the SPNs.

Once established that these inputs do have a true inhibitory effect on SPNs, the next question asked was whether or not the inhibitory synapses, responsible for these effects, were in the spinal cord. This question reduced to that of whether, in acute spinal animals, inhibition could still be obtained. Several authors have suggested, although in the absence of strong supporting experimental evidence (Koizumi et al., 1968; Iwamura, 1969; Coote and Perez-Gonzalez, 1970), that central sympathetic inhibition is brought about by inhibition of a medullary facilitatory center.

Further, it was asked whether the inhibitory nerve endings producing the effects studied in these experiments could not be located on the membrane of the SPN itself. There have been some suggestions (Sato and Schmidt, 1966; Franz, 1966; Franz et al., 1966) that some of the sympathetic inhibitions could be of the presynaptic type. This question led to experiments testing the excitability of the SPN by means of Na glutamate released from a micropipette and of antidromic stimulation.

Finally, other properties of the various inhibitory effects were studied, including spatial summation, occlusion and the effects of drugs, in order to see whether they were compatible with the view that all inhibitory effects were relayed through a common inhibitory pathway or that each input has its own private path to the SPN.

II. METHODS

The data presented below were obtained in a total of 94 cats, of either sex, weighing from 2.5 to 4.5 kg. Eighty-five cats were anesthetized with an initial intraperitoneal or intrathoracic (Lumb, 1963, p. 194) dose of sodium pentobarbital (30 mg/Kg body weight). Additional booster doses were administered intravenously when required. Nine cats were decerebrated under ether, the ether being discontinued upon completion of the surgery.

(i) Surgical Preparation

The initial surgery consisted of exposing and cannulating the trachea for the administration of artificial respiration, a femoral artery for blood pressure recording and a femoral vein for intravenous injections. In the preparations undergoing decerebration, the vertebral and common carotid arteries were permanently occluded under ether anesthesia. This was followed by removal, through a craniotomy, of the forebrain anterior to the tentorium cerebelli. The occlusion of the carotid and vertebral arteries always induced a transient increase in mean arterial blood pressure of 45 mm Hg amplitude on the average and lasting from 5 to 10 minutes.

For the preparation of the spinal animals, complete section of the spinal cord was performed, at high cervical levels (C_2 or C_3) with a scalpel blade. In the initial experiments of this series it was observed that section of the cord caused a transient increase in mean

arterial blood pressure of 50 mm Hg amplitude on the average (decaying to a steady low value in up to 5 minutes) with the occasional occurrence of slow, regularly repeating, oscillations superimposed on the descending limb. Some of these pressor transients were up to 100 mm Hg in amplitude. The mechanism of the pressor response is presumably excitation of descending excitatory pathways by the injury currents set up at the site of section. In the later experiments, the region of the cord to be sectioned was infiltrated with a 2% solution of procaine or transiently cooled. Both procedures prevented the pressor responses associated with sectioning the spinal cord. All electrical recording in spinal animals was performed 2-12 hours after spinal section.

The dorsolateral surface of the spinal cord, usually between C-2 and T-4, was exposed by laminectomy. The dura was reflected and pinned back with small hooks. The exposed cord was bathed in warm paraffin oil or saline. The animal was clamped to a rigid frame at several points prior to electrical recording. Bilateral pneumothorax was performed and a neuromuscular blocking agent (gallamine triethiodide, 10 mg/Kg) was administered intravenously. Positive pressure artificial respiration was then applied. The purpose of these steps was to limit movements of the spinal cord. Every half hour or so, the preparations were hyperventilated (for a few breaths) in order to prevent atelectasis (Collier and Mead, 1964). Rectal temperature was measured and maintained between 35 and 37^{o}C by means of a heating lamp. The temperature of the pool of oil or saline

covering the exposed portion of the spinal cord was measured and generally found not different from the simultaneously recorded values of rectal temperature.

The cervical sympathetic and vagus nerves in the neck, the median and ulnar nerves at the elbow, the sciatic nerve in the thigh, and the splanchnic nerve in the thorax, were dissected from their surrounding connective tissues and prepared for stimulation. The dorsal surface of the caudal medulla oblongata was exposed at the level of the obex by incision and reflection of the atlanto-occipital membrane, followed by removal of the occipital bone up to the level of the tentorium. The dura-arachnoid membrane was then sectioned and reflected, and the floor of the fourth ventricle was exposed by suction of the overlying cerebellum. The exposed portion of the medulla was covered with a thin layer of cotton soaked in paraffin oil.

(ii) Stimulating Technique

Antidromic excitation was used for identification of the sympathetic preganglionic neurons. The cervical sympathetic nerves, which contain the axons of the SPNs of spinal cord segments T-1 to T-5 (Geohegan et al., 1941; Foley and Schnitzlein, 1957), were mounted on buried bipolar silver electrodes. The stimulating pulses were obtained from a type 161 Tektronix square-pulse generator and were delivered to the preparation through a transformer. In later experiments, stimulating pulses were obtained from a Grass S88 stimulator through a stimulus isolation unit (SIU 4678). In some experiments paired antidromic

stimuli were given through the same electrode using two Tektronix 161 square-pulse generators and an operational amplifier (Heath EUW 19A) interposed between the output stage of the stimulators and the preparation. In later experiments, the stimulus pairs were obtained from a Grass S88 stimulator through a stimulus isolation unit (SIU 4678). For peripheral nerve stimulation, the central cut end of the ulnar, median, sciatic, splanchnic and vagus nerves were mounted on buried bipolar silver electrodes or on pairs of silver electrodes in pools of warm paraffin oil. For medullary stimulation a co-axial stainless steel electrode, of 0.5 mm tip diameter (Kopf) was inserted with a micromanipulator 1-1.5 mm below the dorsal surface of the medulla in the area outlined in Fig. 1. For stimulation of descending fibres in the lateral funiculus of the spinal cord, a similar electrode was inserted in the spinal cord at a level between C-2 and C-4 just lateral to the line of dorsal rootlet insertion and advanced into the lateral column to a depth of approximately 1.5 to 2.0 mm.

(iii) Recording and Analysis

Glass microelectrodes of 2-4 microns tip diameter and filled with saturated NaCl solution were used to record extracellularly the spike activity of the neurons. These micropipettes had a DC resistance between 0.3 and 0.8 megohms. In some experiments, double-barrel glass micropipettes, filled with 0.5 M Na glutamate and with saturated NaCl were used (Curtis et al., 1960; Krnjević and Phillis, 1963). The

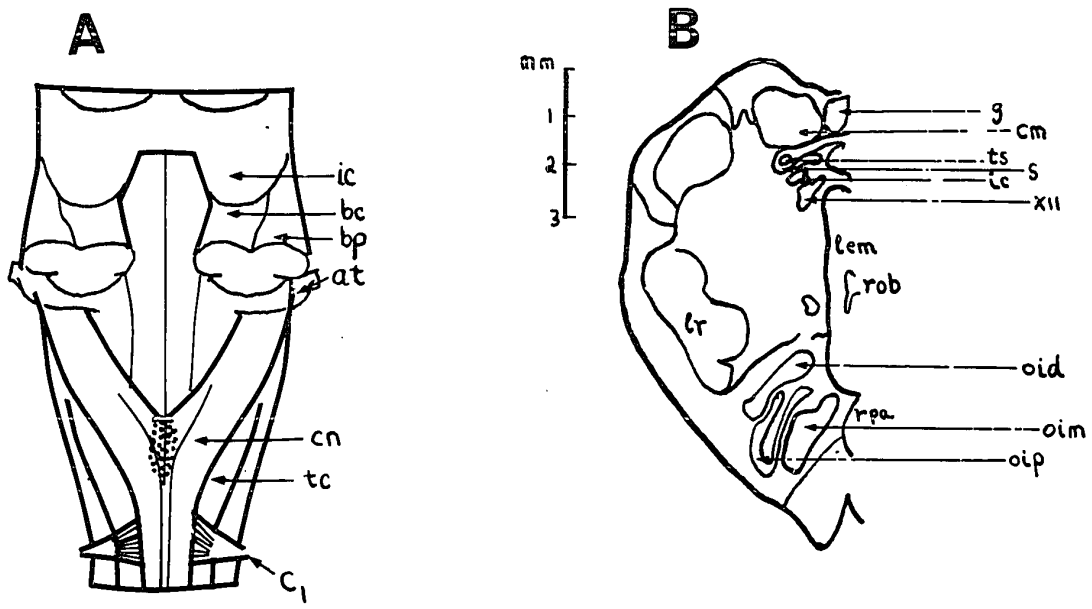

Figure 1. Diagrammatic representation of the dorsal surface (A) and a cross-section (B) of the brainstem 1.0 mm caudal to the obex with the cerebellum removed. Stippled area in A represents the region wherein the stimulating electrode was placed.

Legend:

ic: inferior colliculus
bc: brachium conjunctivum
bp: brachium pontus
at: auditory tubercule
cn: nucleus cuneatus
tc: tuberculum cinereum
C_1: first cervical nerve
g : nucleus gracilis
cm: nucleus cuneatus medialis
ts: tractus solitarius
s : nucleus tractus solitarius
ic: nucleus intercalatus
XII: nucleus nervi hypoglossi
lem: medial lemniscus
rob: nucleus raphe obscurus
oid: nucleus olivaris inferior accessorius dorsalis
rpa: nucleus raphe pallidus
oim: nucleus olivaris inferior accessorius medialis
oip: nucleus olivaris inferior principalis
lr: nucleus lateralis reticularis

Adapted from Alexander, 1946, p. 208 and Crill and Reis, 1968, p. 273.

resistances were of the order of 10 and 5 megohms, respectively. The pipette was advanced inside the cord with a remotely controlled hydraulic microdrive (Burns, 1961) supported by a Narishige micromanipulator. Extracellular potentials were recorded between the tip of the pipette and an indifferent electrode in the neck muscles. The signals from the pipette were led through a cathode follower and AC pre-amplifier (Grass-P5) into a tape-recorder (Teac-R-1000) for permanent storage and into an oscilloscope (Tektronix 502), storage oscilloscope (Tektronix) and loudspeaker for display. Systemic arterial pressure was routinely recorded by means of a Statham strain gauge and Gilson polygraph. All records shown in this thesis were obtained by playing back the magnetic tapes storing the original experimental data into a computer (Burns et al., 1965) and into an oscilloscope for photography. The post-stimulus histograms compiled by the computer were used for the determination of latency and duration of the inhibitory events (see Fig. 2 for example).

(iv) Antidromic Identification of Sympathetic Preganglionic Neurons

A response, time-locked to the stimulus to the cervical nerve, was considered as being from a sympathetic preganglionic unit when it fulfilled the following criteria: i) the response was a single spike which appeared in an all-or-none fashion with respect to stimulus intensity; ii) the latency of the response, measured from the stimulus artefact, was constant and compatible with the latency

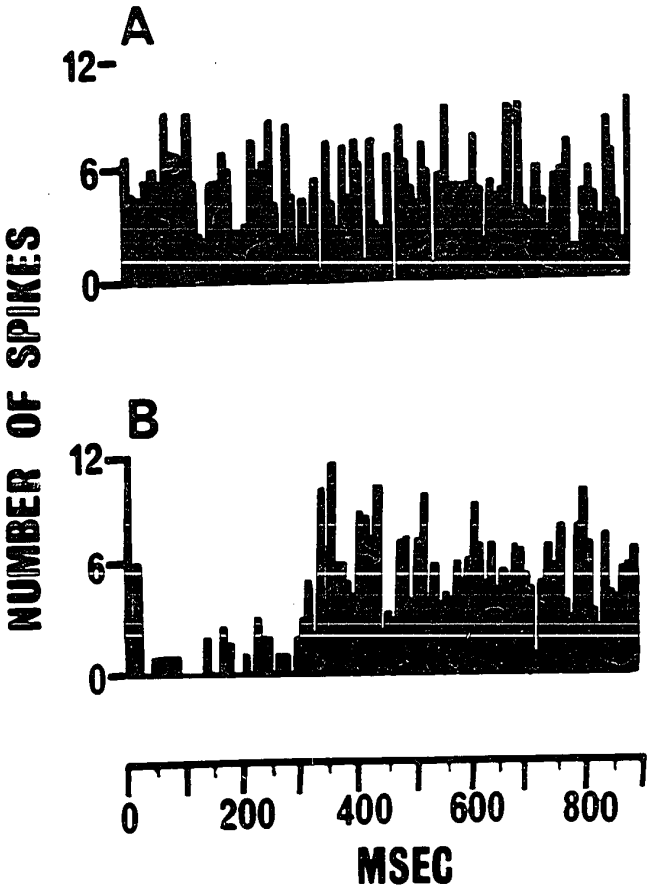

Figure 2. Example of post-stimulus histogram (PSH). A: control background activity. B: sciatic nerve stimulation (50 shocks at 0.2/sec). Plots show number of spikes recorded in 45 successive time bins of 20 msec duration. First bin in B, and in all PSHs shown in this thesis, contains stimulus artefacts and is saturated. Same duration of record analysed in A and B. Computer sweep triggered by an external pulse in A, and by stimulus to sciatic nerve in B.

expected if conduction had been in B-fibres (Grundfest, 1939) and iii) the response could be made to collide with either spontaneous (Paintal, 1959; Bishop et al., 1962; Darian-Smith et al., 1963; Polosa, 1966) or reflexly evoked spikes (Jänig and Schmidt, 1970; Dart, 1971). Antidromic and orthodromic spike responses were considered to be generated by the same unit when they had the same amplitude and could be made to collide (Jänig and Schmidt, 1970). Arguments in support of the assumption that recording was from the soma-dendritic region of the neurons were presented previously by Polosa (1966).

III. RESULTS

(i) Blood Pressure Levels

The mean blood pressure at the start of unit recording was 120 mm Hg on the average (range 75 to 160) in the intact animals. In the spinal animals the mean blood pressure at the start of unit recording was 70 mm Hg on the average (range 40 to 110).

(ii) Excitatory Responses of Sympathetic Preganglionic Neurons

(a) Antidromic Firing

The latencies of the antidromic spike responses of the units ranged from 6 to 90 msec, with a mean of 14 msec. These latencies correspond to approximate conduction velocities of the axons ranging from 10.0 to 0.7 m/sec respectively, with a mean conduction velocity for the entire sample of 4.9 m/sec. Figure 3 is a histogram of the frequency distribution of axonal conduction velocities calculated for a sample of 265 units. These characteristics of the units appear to be in agreement with those of SPNs from the same and from other thoracic cord segments reported previously (Fernandez de Molina et al., 1965; Hongo and Ryall, 1966; Polosa, 1967; Wyszogrodski, 1970; Jänig and Schmidt, 1970).

(b) Background Activity in Intact and Spinal Cats

In the intact anesthetized animals, 34% (73 out of 213 units) of the total population of SPNs observed displayed background activity, i.e. discharged spikes in the absence of any intentionally applied

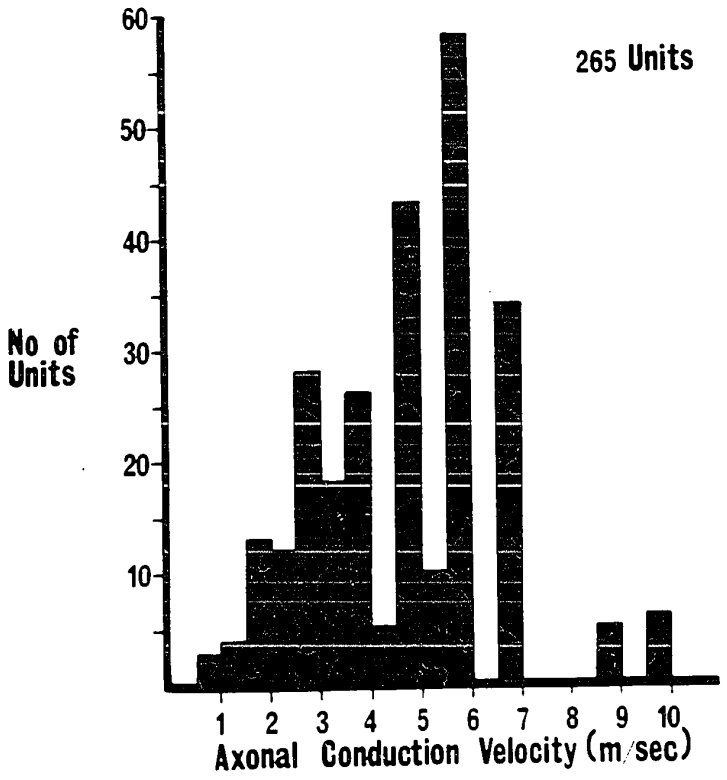

Figure 3. Histogram of axonal conduction velocities of 265 SPNs. Ordinate: number of units. Abscissa: conduction velocity in m/sec. The conduction velocities were calculated by dividing the latency of the antidromic spike into the estimated conduction distance (taken as the linear distance between stimulating and recording sites).

stimuli other than those arising from the standard preparation procedures. The discharge patterns of these spontaneously active neurons could be labelled as "regular", "bursty" and "irregular", adopting a terminology previously used by Polosa (1968) and Wyszogrodski (1970). The "regular" pattern is characterized by an interspike interval which is fairly constant in duration (Fig. 4A). The "irregular" pattern is characterized by the large variability of the interspike interval (Fig. 4B and C). The "bursty" pattern is characterized by regularly repeating groups of spikes separated by periods of inactivity (Fig. 4 D and E). The burst usually consists of 2-8 spikes with the shortest interspike interval usually not less than 100 msec. Approximately 60% of the active units showed the "irregular" pattern of discharge, 30% the "bursty" pattern and 10% the "regular" pattern. The mean rate of discharge of the spontaneously active units ranged from 0.2 to 5.0 spikes/sec, with a mean of 1.5 spikes/sec, (Fig. 5A). Similar values have been reported for the spontaneous discharge rate of SPNs from the same and other spinal cord segments (Hongo and Ryall, 1966; Polosa, 1967; Wyszogrodski, 1970).

In the spinal animal, background activity was less frequent, being observed in 19% (34 out of 176 units) of the total SPN population. Discharge patterns in the spinal animal were mainly "irregular" (90% of the population). The remaining 10% had the "regular" discharge pattern. No "burst" units were found. The rate of discharge of spontaneously active units in this preparation ranged from 0.3 to 4.3 spikes/sec with a mean of 1.2 spikes/sec (Fig. 5B).

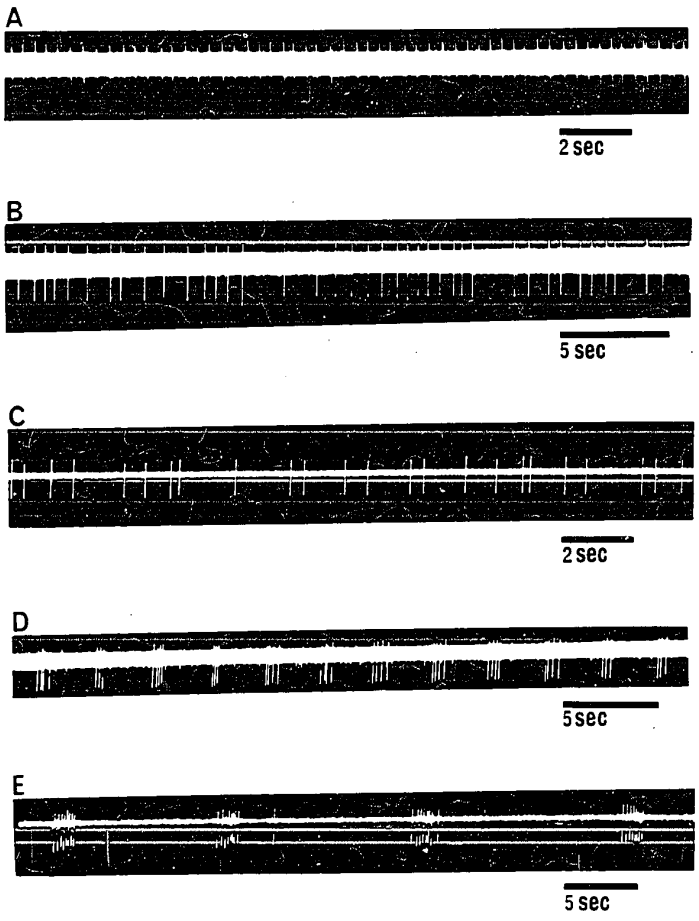

Figure 4. Patterns of background activity of SPNs. A: unit with a "regular" pattern of discharge. Note the constancy of the interspike interval. B, C: two units with "irregular" pattern of discharge. Note the large variability in the duration of the interspike interval. D, E: two units with "bursty" pattern of discharge.

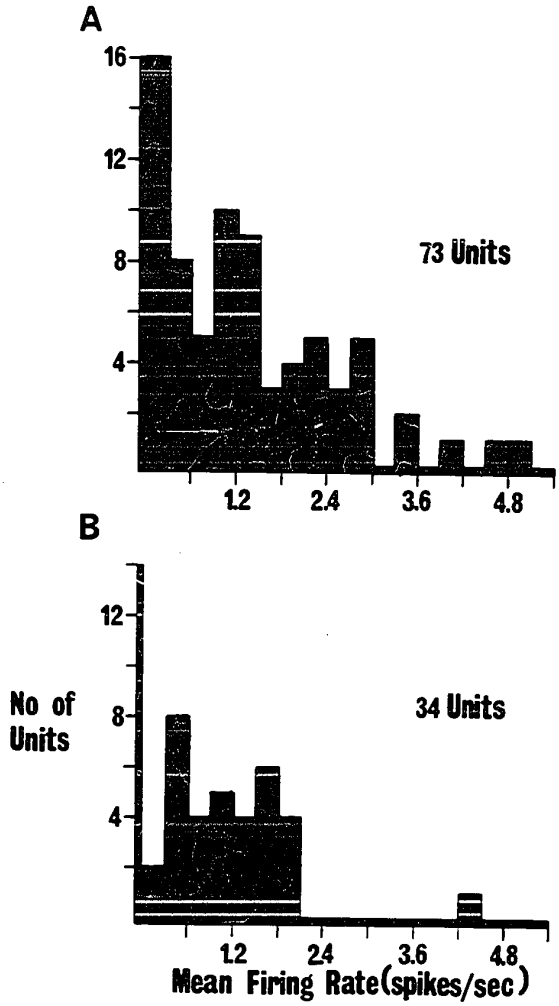

Figure 5. Frequency distribution of mean firing rates of SPNs displaying spontaneous activity. A: cats with intact neuraxis. B: acute spinal cats. Ordinate: no. of units. Abscissa: mean firing rate (spikes/sec).

(c) Glutamate-Evoked Firing

Seventy-five units both in intact and spinal preparations, which did not fire spontaneously, were evoked into activity by the iontophoretic application of Na glutamate. The patterns of glutamate-evoked discharge could also be classified as "irregular" (Fig. 6A), "regular" (Fig. 6B) or "bursty" (Fig. 6C). With increasing glutamate ejection, the cells' firing rate increased, and their firing pattern became more "regular", as shown by the sequence in Fig. 6C-E. This unit was fired by glutamate and displayed a bursty pattern. With increasing glutamate release the number of spikes within the burst and the burst duration increased (Fig. 6C and D). With yet increasing amounts of glutamate the cell started to fire throughout the inter-burst interval (Fig. 6E). A problem which frequently occurred with sustained or increasing glutamate ejection was a progressive decrease in spike amplitude until the spikes could become undetectable. Decreasing the release of glutamate caused the reappearance of the spikes. This behaviour could have been due to depolarization block. A similar phenomenon has been seen with SPNs using DL-homocysteic acid and has received similar interpretation (Hongo and Ryall, 1966; DeGroat and Ryall, 1967). The maximum firing rate at which these SPNs could be driven by glutamate without the production of a depolarizing block was 15-20 spikes/sec. In most experiments the glutamate ejection was adjusted so as to obtain firing rates comparable to those observed in spontaneously firing SPNs. In this way one could avoid the complication of depolarization block, maintain the firing of the cell at a

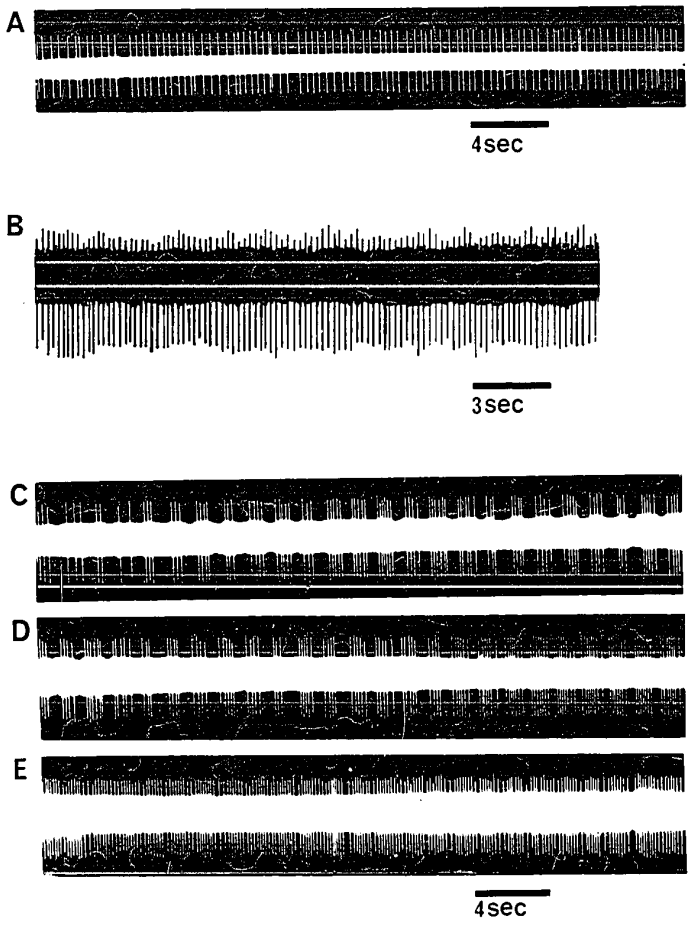

Figure 6. Patterns of glutamate-evoked background activity of SPNs. A: unit with an "irregular" patterns of discharge. Note the large variability in the duration of the interspike interval. B: unit with a "regular" pattern of discharge. Note the constancy of the interspike interval. C,D,E: conversion of the "bursty" pattern of discharge (C and D) characterized by groups of spikes separated by periods of inactivity, into a sustained pattern of discharge (E). C,D, and E are parts of a continuous record from the same unit.

relatively steady level for a long enough time and enable a comparison of the data obtained from spontaneously active and glutamate evoked cells.

(d) Injury Discharge

Another form of induced activity was the discharge resulting from mechanical insult by the microelectrode due to movement of the electrode or of the surrounding tissue. The criteria for attributing the discharge of a cell to mechanical insult were: i) the sudden appearance of firing in a previously quiescent, antidromically identified, unit during movement of the electrode, ii) the dependence of firing rate on electrode position and iii) occasionally the abrupt change in shape and/or polarity of the spike (e.g. appearance of notches, fractionation of the spike into two components, occurrence of positive first phase spikes) with electrode movement. This type of discharge often produced the fastest firing frequencies of the cell (Fig. 7A). For a relatively mild but prolonged injury some cells fired steadily with constant interspike intervals. More severe injury caused the cell to become inexcitable (Fig. 7B and C).

(e) Synaptically-Evoked Firing

SPNs could be made to fire orthodromically by single shock stimulation of the sciatic, ulnar, median, splanchnic and cervical vagus nerves or of sites in the medulla and spinal cord. The average latency of the reflex firing evoked by sciatic nerve stimulation in the intact animal, measured from the stimulus artefact was 130 msec (range 40-300, 42 units). There were two peaks of excitation, one in

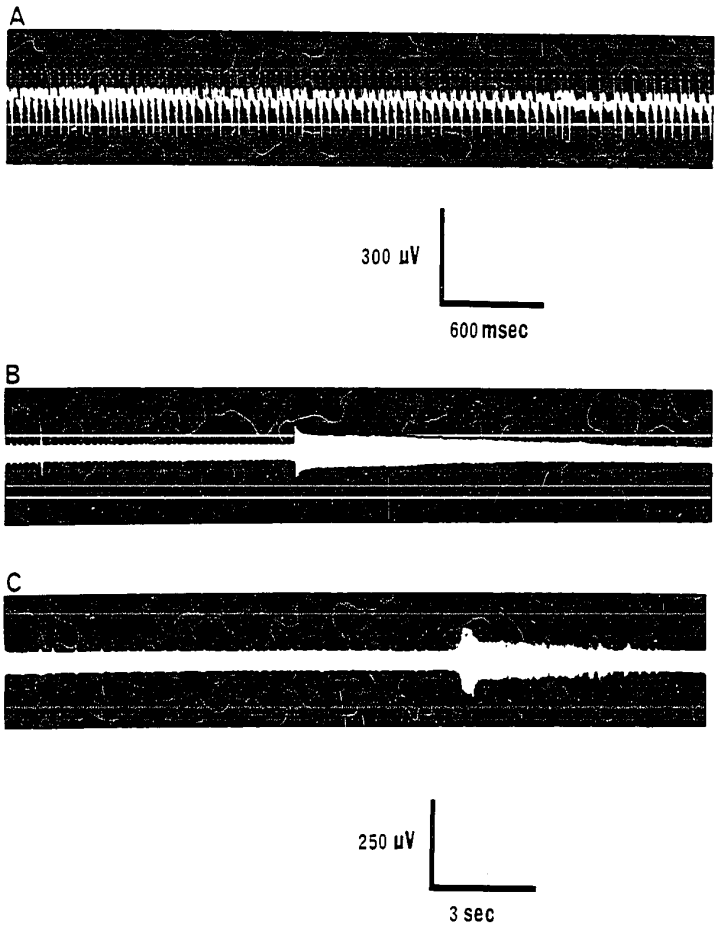

Figure 7. Injury discharge of SPNs. A,B, and C are from three different SPNs. Calibrations are the same in B and C. Note that for the units displayed in B and C, increasing injury causes the cell to greatly increase its firing rate after which it becomes inexcitable.

the 50-150 msec range and the other in the 200-250 msec range. Ulnar and median nerve stimulation evoked reflex discharges with an average latency of 80 msec (range 10-180, 13 units). For the splanchnic nerve the average latency for reflex excitation was 105 msec (range 60-220, 14 units), for the medulla 75 msec (range 5-250, 15 units) and for spinal cord stimulation, 30 msec (range 10-45, 3 units).

The firing index, i.e. the ratio of the number of spikes evoked to the number of stimuli, was 0.9 for the sciatic nerve (range 0.2-2.3, 18 units), 1.0 for the ulnar and median (range 0.2-2.8, 22 units), and 0.8 for the splanchnic (range 0.1-2.0, 10 units). For supraspinal inputs, the firing index was 1.4 (range 0.6-2.4, 12 units) for medullary stimulation, 0.7 (range 0.4-0.9, 3 units) for spinal cord stimulation, and 1.2 (range 0.3-3.3, 16 units) for vagal stimulation. From these figures it would seem that supraspinal inputs had a more powerful excitatory effect than spinal ones.

For the population of cells which were spontaneously active or induced into activity by glutamate it was seen that stimulation within the medulla excited the greatest proportion of the SPNs tested (56% - 15 of 27 units) while the splanchnic was least effective (26% - 18 of 62 units). The remaining inputs all excited between 30-40% of the population. In addition, in several units which were inactive, a subthreshold increase in excitability (i.e. not leading to spike generation) could be detected on occasion by antidromic testing (see p. 50).

(iii) Inhibitory Responses of Sympathetic Preganglionic Neurons

A. Inhibition from Spinal Inputs

(a) Inhibition by Afferents in Limb Nerves

Intact Animals

The effects of limb nerve stimulation (sciatic, ulnar or median) was tested on a population of 200 SPNs. The activity of 30 SPNs with background firing (either spontaneous or glutamate induced) was depressed by single shocks to the sciatic, ulnar or median nerve. This depression of activity was not preceded by any increase in firing rate, i.e. excitation, and hence the underlying process must be an inhibition or disfacilitation and not a post-excitatory depression. This depression of background firing lasted several hundred milliseconds (Fig. 8 and 9). Inhibition of the glutamate-evoked discharge by single shocks could be seen even when activity was of high repetition rate (10-15 spikes/sec). For one additional unit, inhibition of firing could only be evoked by tetanic stimulation. This probably indicates that, in this unit, a considerably larger temporal summation was required for evoking an inhibitory effect. As will be reported later (p. 55), this requirement for temporal summation was much more pronounced in the acute spinal animal. Any unit which could be inhibited by single shocks could also be inhibited by tetanic stimulation.

Inhibition of SPNs could also be demonstrated against the background of injury discharge. Fig. 10A and B, shows a cell which was inactive (at the beginning of the record) but after a small movement of the electrode began to fire with a mean firing rate as high as

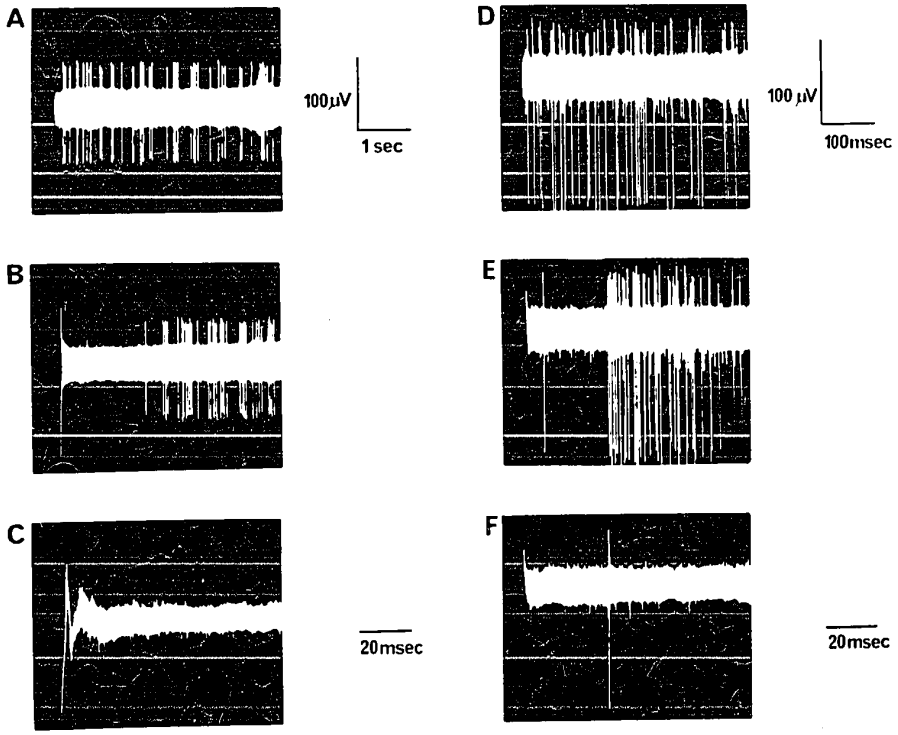

Figure 8. Inhibition of the spontaneous firing of SPNs by single shocks to the ulnar (B,C) and sciatic (E,F) nerves in the intact cat. A,B,C: data from one SPN and D,E,F from another SPN. A,D: control. Superimposed sweeps (33 in A and 50 in D) triggered by any spontaneous spike. B: shocks to the ulnar nerve (5V., 0.5 msec). 33 superimposed sweeps triggered by the stimulus pulse. Note absence of spikes for 2 sec after stimulus. E: shocks to the sciatic nerve (20 V., 0.5 msec,)50 superimposed sweeps triggered by the stimulus pulse. C and F: same as B and E respectively but at faster sweep speed to show absence of excitation. Uppermost time calibrations apply to A,B and D,E respectively.

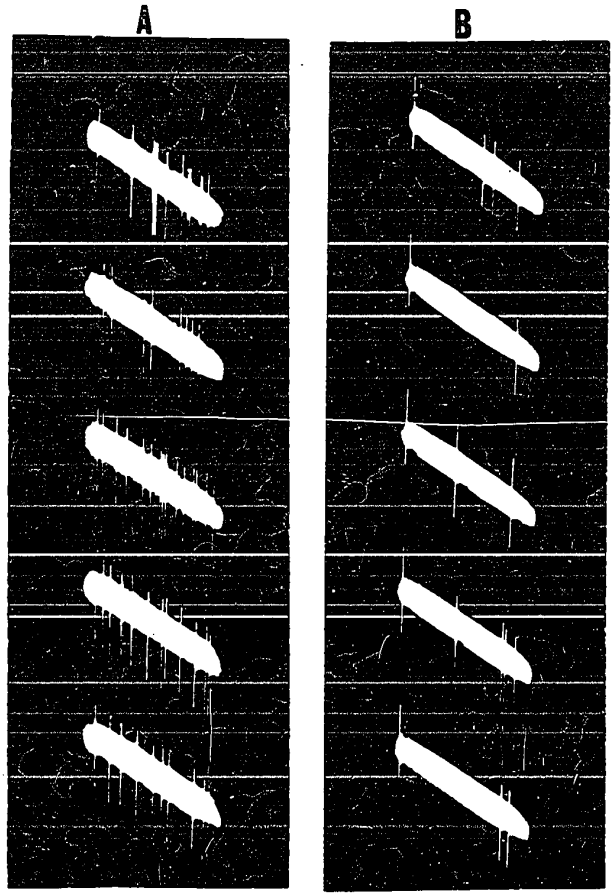

Figure 9. Inhibition by single shocks to ulnar nerve of SPN firing evoked by the steady release of glutamate. A: control consecutive single sweeps. Average number of spikes per sweep is 11. B: shocks to ulnar nerve (10 V., 0.5 msec). Sweeps triggered by stimulus artefact. Average number of spikes per sweep is 2.

15 spikes/sec. Repetitive stimulation of the ulnar nerve at 1/sec (dots) produces an inhibition of discharge after every stimulus. Fig. 10B shows that, probably due to increasing injury, there was a high frequency burst after which the cell becomes inexcitable. Fig. 10E shows, for the same unit, superimposed sweeps of the first 100 msec following the stimulus. There is no early excitation and hence the suppression of firing was due to inhibition and not to a post-excitation depression. Fig 10C shows another unit which was also firing as a result of injury at a mean firing rate as high as 24 spikes/sec. A similar suppression of activity which could not be attributed to a post-excitatory depression (Fig. 10F) occurred as a result of ulnar nerve stimulation at 1/sec (Fig. 10C and D).

Antidromic firing evoked by single shocks to the cervical nerve was not blocked by single or repetitive stimulation of limb nerves. However, when antidromic shock pairs were given, a decreased probability of antidromic invasion during limb nerve stimulation was detected in a few cells. The individual stimuli in the pair (of intensity 3-5 times the unit threshold) were spaced so that the testing antidromic spike would be reaching the cell body during the early recovery phase following the conditioning firing (conditioning-test interval of the order of 6-8 msec) and would be on the verge of, or actually failing to, invade the cell body in a proportion of the trials. When failures occurred, they were not due to absolute refractoriness of the axon because the latter could still be excited at shorter conditioning-test intervals than those at which the soma-

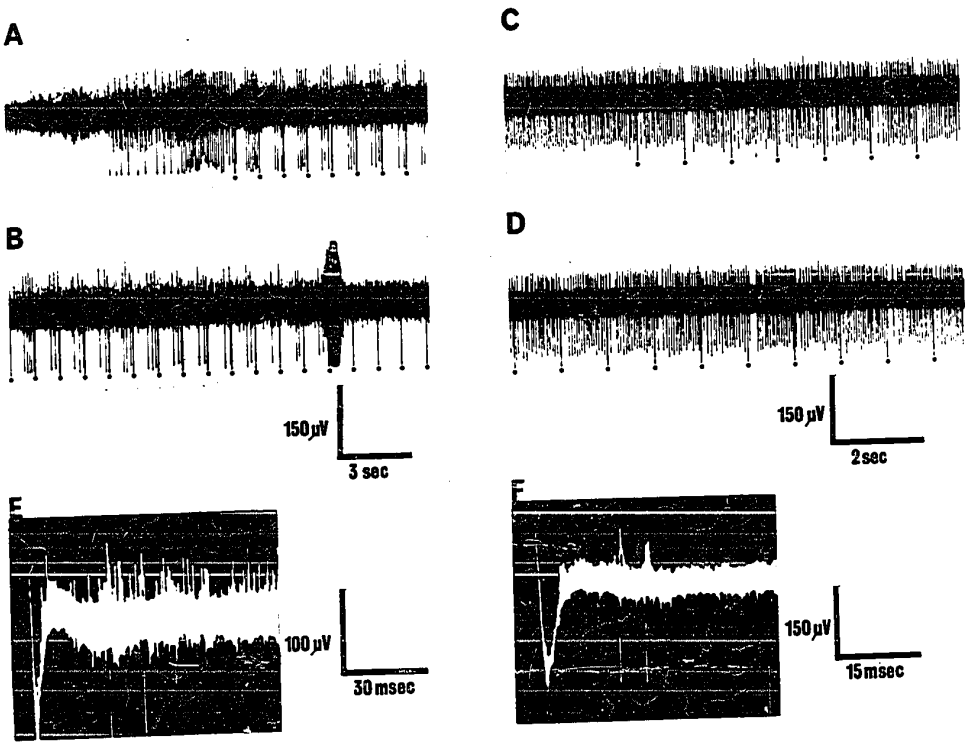

Figure 10. Inhibition of injury discharge by somatic afferents. A, B and E: quiescent SPN. At beginning of A, unit starts to fire, as a result of small electrode movement. Discharge is inhibited by shocks to the ulnar nerve (15V., 0.5 msec) given at 1/sec (stimulus artefacts marked by dots). Towards end of B, unit gives burst of high frequency discharge and stops firing. In E, 20 superimposed sweeps triggered by stimulus to show absence of excitation. C, D and F: another SPN, firing as a result of electrode movement. Discharge inhibited by shocks to the ulnar nerve (8V., 1.0 msec) at 1/sec (stimulus artefacts marked by dots). In F, 15 superimposed sweeps triggered by stimulus artefact, to show absence of excitation.

dendritic spike began to fail (cf. Brock et al., 1953; Polosa, 1966, p.30). In addition, the previously reported observation (Wyszogrodski, 1970: Fig. 14) that the number of failures can decrease during sciatic nerve stimulation presumably as a result of subthreshold EPSP generation on the SPN's membrane shows that these failures are due to axon-soma block and not to a failure of axonal excitation or conduction, because the latter would not be expected to improve with sciatic nerve stimulation. Fig 11 shows a quiescent unit in which the probability of antidromic invasion decreased during tetanic stimulation of the ulnar nerve. With an interval of 6 msec between conditioning and testing antidromic shocks, the likelihood of obtaining a response to the test antidromic stimulus was 100%. During a period of tetanic stimulation of the ulnar nerve at 10 cps the likelihood of obtaining a response to the test stimulus decreased to zero.

Concerning the afferent fibre types responsible for the inhibition, the intensity of stimulation necessary for evoking a just detectable inhibitory effect ranged in different units from 4.1T (threshold for I A. afferents) to 55.0T, on the average 15T (11 units). From these figures it can be inferred that group III and possibly some group II afferents (Brock et al., 1951; Eccles and Lundberg, 1959; Rosenberg, 1970) were responsible for the inhibition. These same fibre groups are known to evoke blood pressure falls in anesthetized cats when stimulated repetitively at low frequency (Laporte and Montastruc, 1957; Laporte et al., 1960; Skoglund, 1960; Coote and Perez-Gonzalez, 1970; Wyszogrodski, 1970). With the exception of the experiments in which the relation of the inhibition to

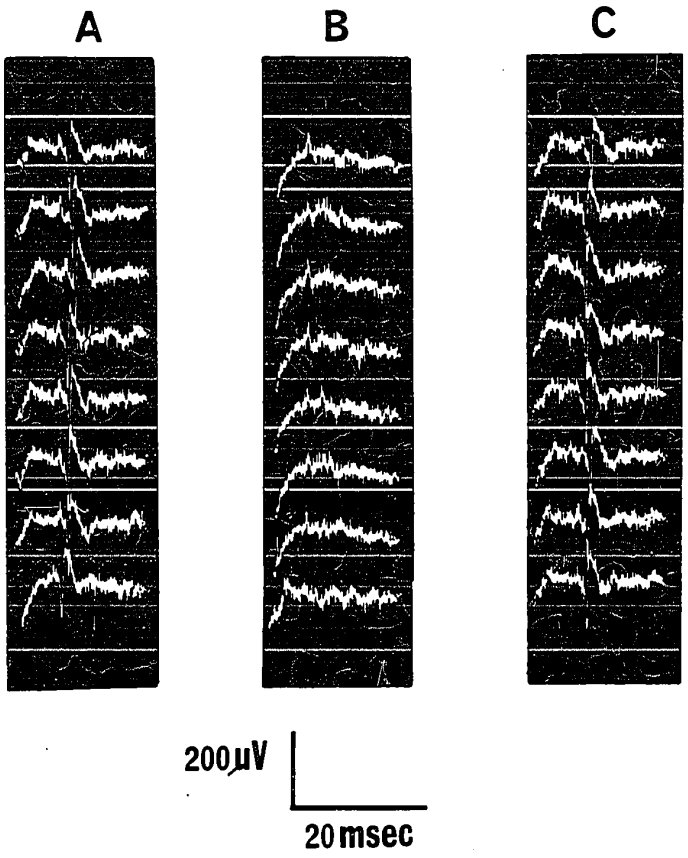

Figure 11. Inhibition of antidromic invasion by somatic afferents. Antidromic shock pairs given every 5 sec. Each sweep shows events following test shock only. A and C: controls, before and after B. Spike response is present after each test antidromic stimulus. B: during ulnar nerve stimulation at 10/sec. Spike response to testing antidromic stimulus is absent. There was a slight blood pressure drop (5 mm Hg) during afferent stimulation.

afferent fibre types was specifically investigated, the stimulus intensity used was that which gave the most pronounced inhibitory effects.

Latency and duration of the inhibition of spontaneously active or glutamate-evoked SPNs could be estimated from post-stimulus histograms or from photographic records of several superimposed stimulus triggered sweeps. The latency of the inhibition was on the average 96 msec (range 20 to 240) with sciatic nerve stimulation (20 units) and 48 msec (range 10 to 120) with ulnar and median nerve stimulation (18 units). The frequency distribution of latencies in these samples is shown in Fig 12A and C. The difference in latencies between sciatic and ulnar or median nerves is significant at the 0.001 level and could be attributed to the difference in the distance the afferent volleys have to travel within the spinal cord. The shortest latencies with the ulnar and median nerves suggest pathways with only few synapses. In several units latency could not be estimated with any degree of certainty due to factors such as short duration of contact with the unit, low frequency and high variability of firing, and long durations of inhibition requiring low stimulus repetition rates. For the few units in which satisfactory threshold determinations were available, an estimate of central transmission time was made. Values for conduction velocities were obtained from fibre threshold using the plots published by Eccles and Lundberg (1959) and Rosenberg (1970). Peripheral conduction distance was taken between cathode and either L-6 dorsal root entry zone (for the sciatic nerve)

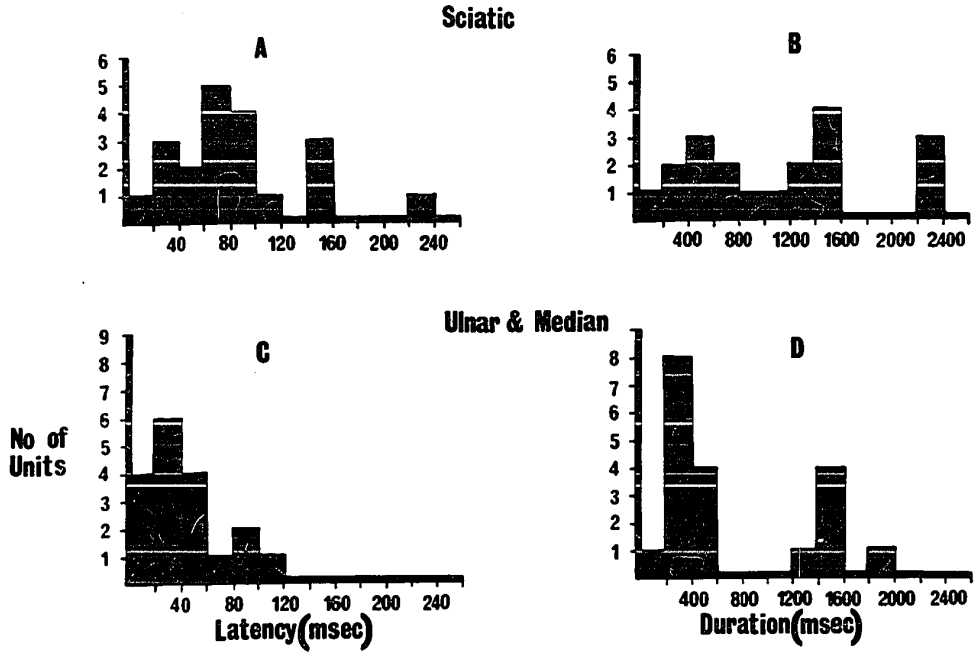

Figure 12. Frequency distribution of latencies (A and C) and durations (B and D) of inhibition for a sample of SPNs in the intact preparation inhibited by single shocks to the sciatic (A and B) or ulnar and median (C and D) nerves. Ordinate: number of units. Abscissa: latency (msec) in A and C, and duration (msec) in B and D.

or T-1 dorsal root entry zone (for the ulnar and median nerves) (Reighard and Jennings, 1901). After subtracting peripheral conduction time, central transmission time ranged from 30 to 93 msec, average 58 msec (4 units) with sciatic nerve stimulation and from 16 to 77 msec, average 31 msec (7 units) with ulnar and median nerve stimulation.

The duration of inhibition, evoked by single maximal stimuli, was, on the average, 1100 msec (range 180 to 2400) with sciatic nerve stimulation (19 units) and 720 msec (range 140 to 2000) with ulnar and median nerve stimulation (19 units). The inhibition was of several hundred msec duration even with threshold stimulus intensity. The frequency distribution of duration of inhibitions in the sample is shown in Fig. 12B and D. A gradation of duration of inhibition could be obtained by varying the intensityof stimulation from threshold to maximal. No appreciable difference in the duration of inhibition was observed, for comparable firing rates, whether spontaneous or glutamate-evoked activity was used as a background for detecting inhibition; e.g. with single supramaximal shocks to the sciatic nerve the duration of inhibition of spontaneous activity (10 units) was 1190 msec on the average (range 180 to 2260) and the duration of inhibition of glutamate-evoked activity (8 units), was on the average 1000 msec (range 240 to 2210).

The proportion of the neuron population, that could be inhibited by somatic afferent stimulation varied considerably from one experiment to the other. On the basis of data from glutamate experiments, in which nearly every cell could be made to fire and could therefore

show presence or absence of inhibition, the proportion was estimated to be between 1/3 and 1/2. The ulnar nerve appeared to be a more powerful inhibitory nerve, for the T_1 SPNs, than the sciatic nerve, as shown by the fact that when both nerves were tested on the same cell, the likelihood of obtaining inhibition from the ulnar was higher than from the sciatic. Out of 18 units, in which either input, or both, had an effect, 9 were inhibited by ulnar nerve only, one by sciatic nerve only and the remaining 8 by both nerves.

Acute Spinal Animals

The effects of limb nerve stimulation (sciatic, ulnar and median) were tested on a population of 151 SPNs in cats whose cervical spinal cord had been sectioned (see Methods, p. 27). Single shock stimulation of these nerves inhibited the spontaneous or the glutamate-evoked activity of 14 units. For 7 additional units a detectable inhibition could only be evoked by tetanic stimulation. In contrast, in the group with intact spinal cord, tetanic stimulation recruited only one additional unit (p. 45). Examples of inhibition of background activity in spinal animals by single shock and tetanic stimulation are given in Fig. 13. These findings demonstrate that the pathway for somatosympathetic inhibition is complete within the spinal cord. Antidromic invasion, tested with double shocks, as described earlier, could also be inhibited in a few units by tetanic stimulation of the limb nerves.

The average latency of inhibition, with single shock stimulation of the sciatic nerve (7 units) was 65 msec (range 40 to 100) and with ulnar or median nerve stimulation (7 units) was 40 msec (range 30 to

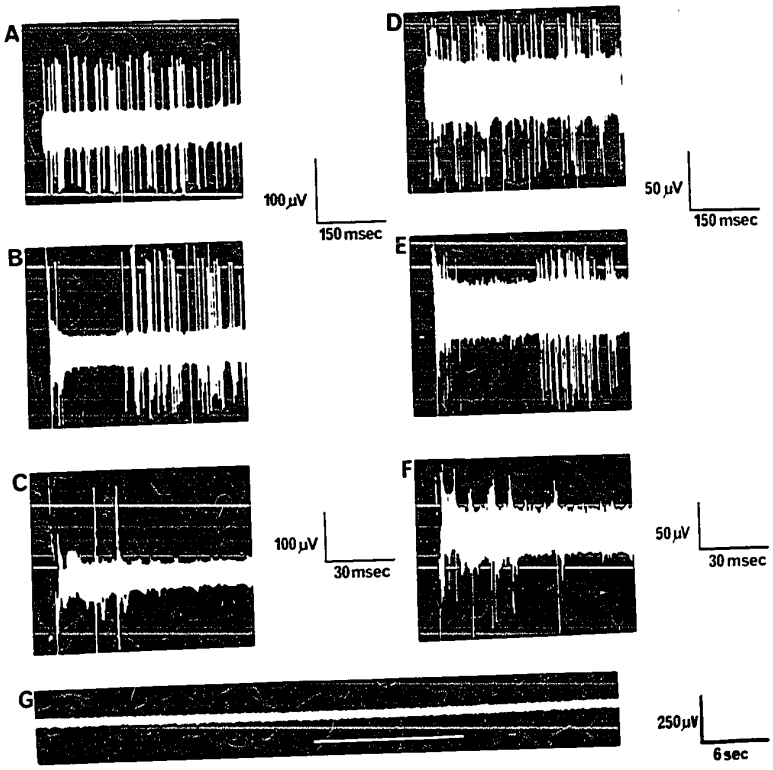

Figure 13. Inhibition by somatic afferents of glutamate-evoked (A-F) and spontaneous (G) activity of SPNs in the acute spinal cat. A and D: controls for two units. 60 sweeps triggered by any spike. B and E: 60 sweeps triggered by shocks to ulnar nerve. C and F: same as in B and E, respectively, but at faster sweep speed to show absence of excitation. G: inhibition by a tetanus (marked by a horizontal line) to sciatic nerve (10/sec). No arterial pressure changes associated with the stimulation. Uppermost calibrations apply to A, B and D, E respectively.

50) (Fig. 14A and C). The average duration of inhibition was 360 msec (range 100 to 480) with sciatic nerve stimulation (9 units), and 265 msec (range 140 to 450) with ulnar or median nerve stimulation (7 units; Fig. 14B and D). When the data for latency and duration are compared with that of the group with intact cord, it appears that the average duration of the inhibition evoked by a single shock is greatly reduced after spinal cord section. There is a total lack of durations greater than 500 msec (compare Fig. 12B with Fig. 14B and Fig. 12D with Fig. 14D). The average latency of ulnar inhibition appears unchanged while that of sciatic inhibition is decreased (see Discussion). The decrease in duration in the spinal animal could be explained by an interruption (anatomical or functional) of those inhibitory pathways connecting somatic afferents to SPNs which have the longest transit times (see Discussion, p.107). The intensity of peripheral limb nerve stimulation necessary for evoking a just detectable inhibition was comparable to that required in the cat with intact spinal cord, suggesting that the same afferent fibre group responsible for inhibition in the intact animal is also responsible for inhibition in the acute spinal animal.

(b) Inhibition by Afferents in the Splanchnic Nerve

Intact Animals

The effects of stimulation of afferents in the splanchnic nerve were tested on a population of 96 SPNs. The activity of 38% (17 out of 45 units) of the SPNs with background firing (either spontaneous or glutamate-evoked) was depressed by single shocks to the splanchnic nerve. This depression of background firing, as in the case of somatic

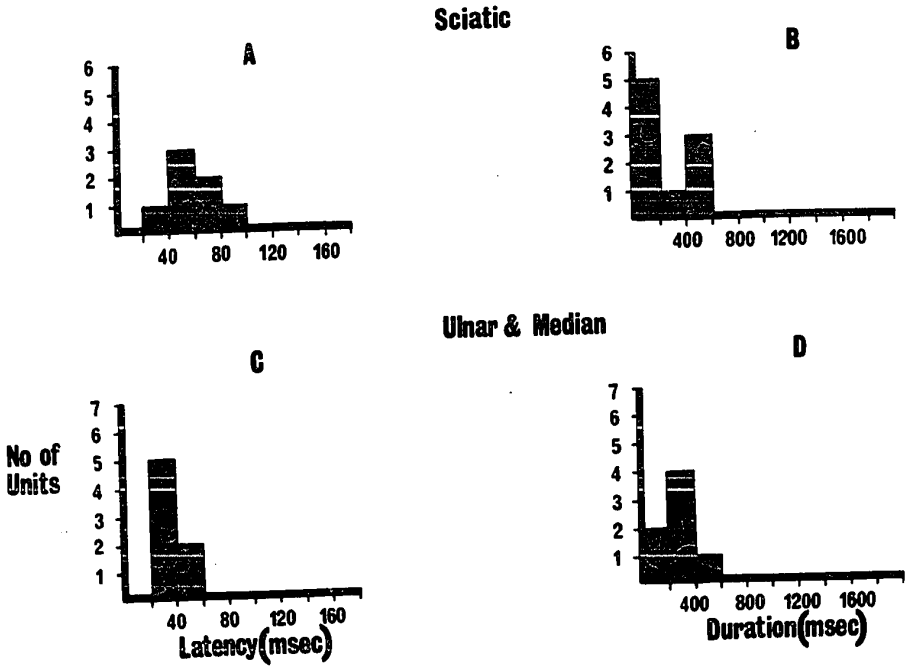

Figure 14. Frequency distribution of latencies (A and C) and durations (B and D) of inhibition for a sample of SPNs in the acute spinal preparation inhibited by single shocks to the sciatic (A and B) and ulnar or median (C and D) nerves. Ordinate: number of units. Abscissa: latency (msec) in A and C, and duration (msec) in B and D.

afferents, lasted several hundred milliseconds (Fig. 15 and 16). Three additional units could only be inhibited by repetitive stimulation of the nerve. Any unit which could be inhibited by single shocks could also be inhibited by tetanic stimulation. Inhibition of SPN firing by splanchnic nerve stimulation could also be demonstrated against a background of injury discharges and occasionally against antidromic firing evoked by C-T shocks applied to the axon as described above (p.48). In the case of the unit shown in Fig. 17, with an interval of 5 msec between conditioning and testing antidromic shocks, the likelihood of obtaining a response to the test antidromic stimulus was approximately 64% (Fig. 17A). During a period of tetanic stimulation of the splanchnic nerve at 15 cps the likelihood of obtaining a response to the test stimulus decreased to 27% (Fig. 17B). The intensity of stimulation necessary for evoking a just detectable inhibition ranged in different units from 8.9 to 16.7 times the threshold for 1A afferents. Thus the afferent fibres responsible for the inhibition appear to belong to group III fibres. The latency of the inhibition from the splanchnic, measured from post-stimulus histograms in 16 units, was, on the average, 71 msec (range 40 to 140). The frequency distribution of latencies in the sample is shown in Fig. 18A. Due to the diffuse pattern of entry of splanchnic afferents into the spinal cord, via a large number of dorsal roots (Mei et al., 1970) and the sympathetic chain (Franz et al., 1966), no estimates of central delay could be made. The duration of inhibition with single maximal stimuli was on the average (17 units) 1070 msec (range 120 to 2100).

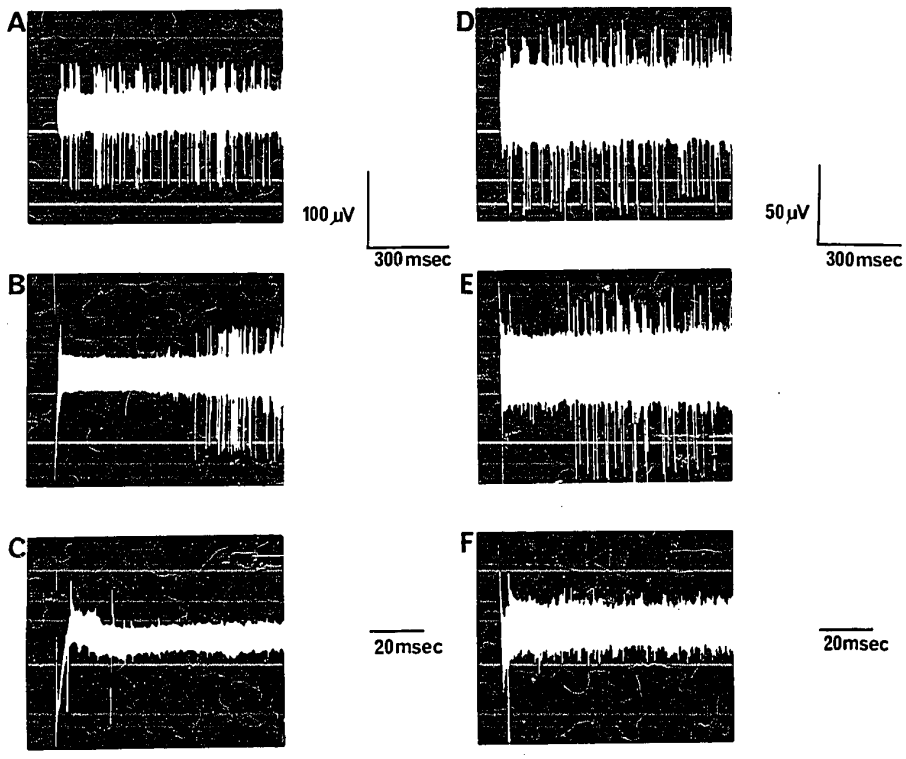

Figure 15. Inhibition by the splanchnic nerve of glutamate-evoked (A-C) and spontaneous (D-F) activity of 2 SPNs in the intact preparation. A and D: controls. 35 superimposed sweeps in A and 10 superimposed sweeps in D triggered by any spike. B and E: 35 and 10 sweeps, respectively, triggered by shocks to the splanchnic nerve. C and F: same as in B and E, but at faster sweep speed to show absence of excitation. Uppermost time calibrations apply to A, B and D, E respectively.

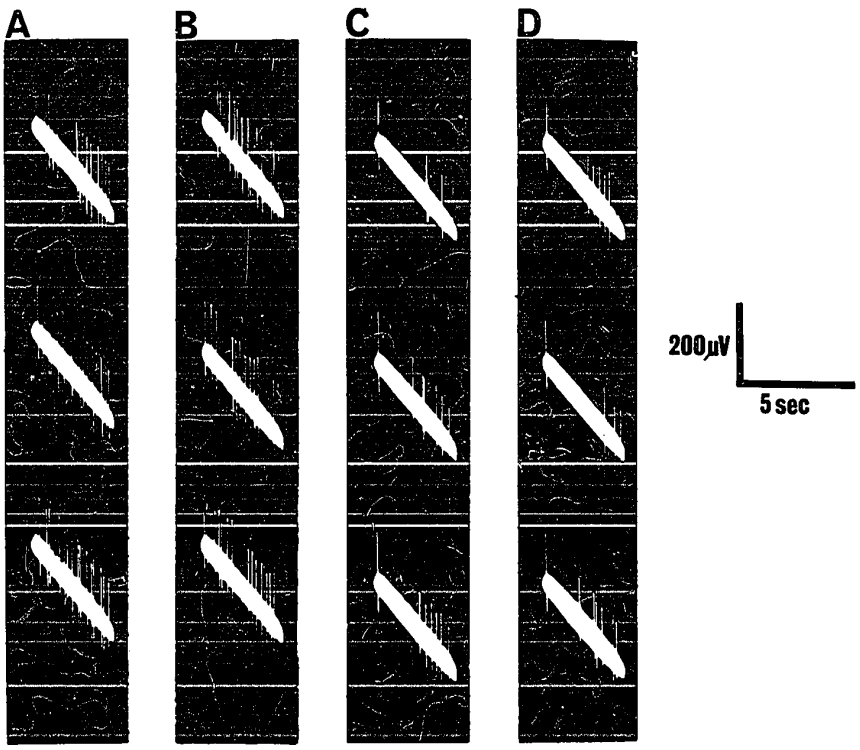

Figure 16. Inhibition by single shocks to the splanchnic nerve of SPN firing evoked by the steady release of glutamate. A and B: control consecutive single sweeps. Average number of spikes per sweep is 14. C and D: shocks to splanchnic nerve (15V., 1.0 msec). Sweeps triggered by stimulus artefact. Average number of spikes per sweep is 5.

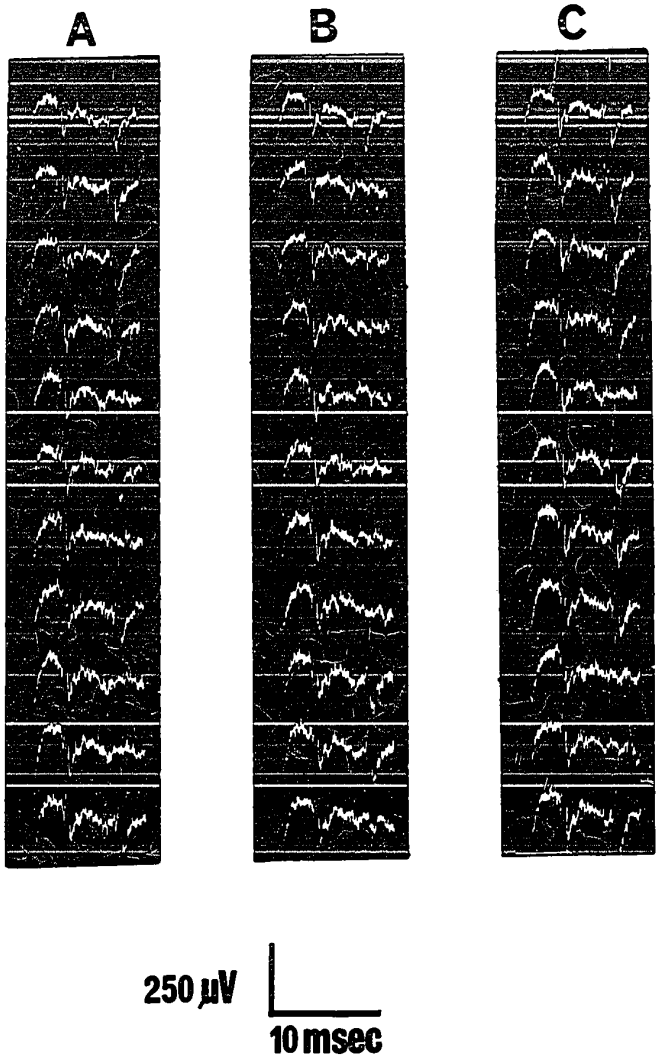

Figure 17. Inhibition of antidromic invasion by stimulation of splanchnic nerve afferents. Antidromic shock pairs given every 5 sec. Each sweep shows the spike response to the conditioning stimulus (first spike; always present) and the spike response following the test stimulus (second spike: present only at times). In A, before splanchnic stimulation, the unit responds to the test stimulus 7 out of 11 times. In B, during splanchnic stimulation at 15/sec, 20 volts and 1.0 msec pulse duration the unit responds to the test stimulus 3 out of 11 times. In C, after splanchnic stimulation was terminated, the number of responses to the test stimulus increased to 8 out of 11. There was no blood pressure change during afferent stimulation.

The inhibition was of several hundred msec duration even with threshold stimulus intensity. The frequency distribution of durations of inhibition in the sample is shown in Fig. 18B.

Acute Spinal Animals

The effect of splanchnic nerve stimulation was tested on a population of 66 SPNs in cats with sectioned spinal cord. The activity of 6 out of 20 units with background firing (either spontaneous or glutamate-evoked) was depressed by single supramaximal shocks to the splanchnic nerve, (Fig. 19). For an additional unit a detectable inhibition could only be induced by tetanic stimulation of the splanchnic nerve. Antidromic invasion, tested with double shocks was inhibited in one case by orthodromic tentanic stimulation of the splanchnic nerve. The average latency of the inhibition, with single maximal shock stimulation (5 units) was 49 msec (range 25 to 100) (Fig. 20A). The average duration of inhibition (6 units) was 290 msec (range 150 to 600) (Fig. 20B). When the latency and duration are compared with those of the group with intact cord, it appears that both the mean latency and the mean duration of the inhibition evoked by a single shock are greatly reduced after spinal cord section. A similar change has been described for somato-sympathetic inhibition (cf. p. 59).

B. Inhibition from Supraspinal Inputs

(c) Inhibition by Afferents in the Cervical Vagus Nerve

The effects of stimulation of afferents in the cervical vagus

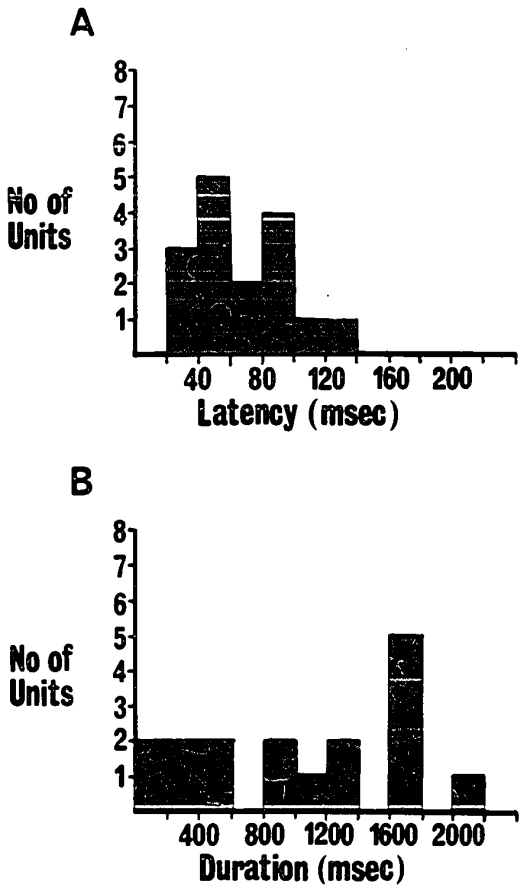

Figure 18. Frequency distribution of latencies (A) and durations (B) of inhibition for a sample of SPNs in the intact preparation inhibited by single shocks to the splanchnic nerve. Ordinate: number of units. Abscissa: latency (msec) in A and duration (msec) in B.

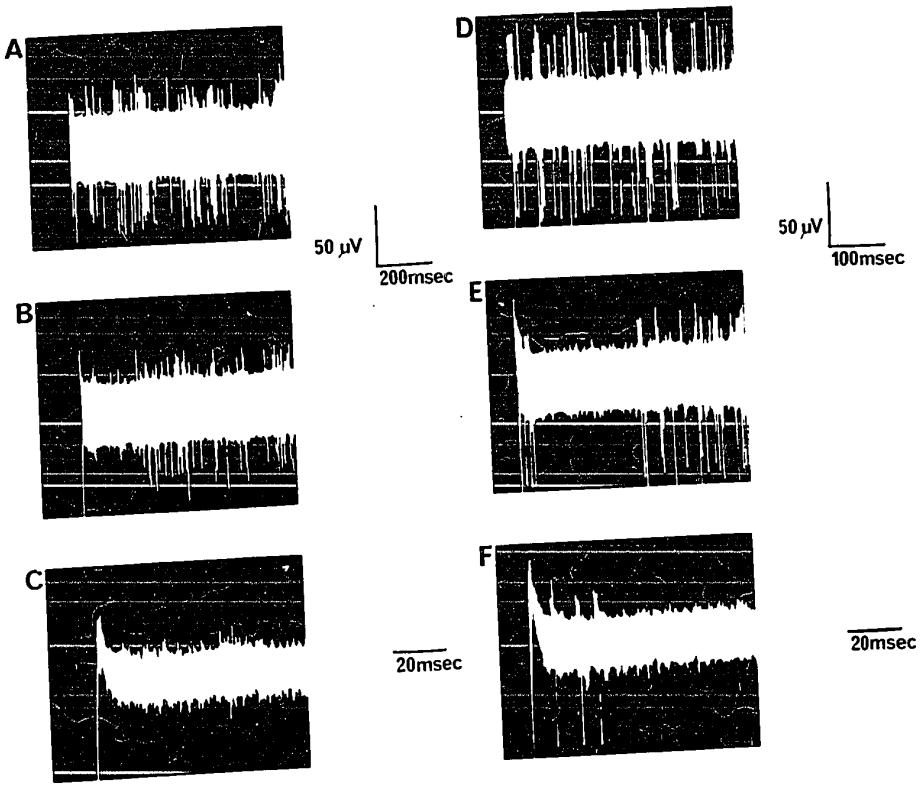

Figure 19. Inhibition of glutamate-evoked activity of SPNs by single shocks to the splanchnic nerve in the acute spinal cat. A, B, C: data from one SPN and D, E, F, from another SPN. A, D: control, superimposed sweeps (23 in A and 40 in D) triggered by any spike. B, E: superimposed sweeps (23 in B, 40 in E,) triggered by shocks to the splanchnic nerve (20V., 0.5 msec in B, and 20V., 1.0 msec in E). C, F: same as in B and E, but at faster sweep speed to show absence of excitation. Uppermost time calibrations apply to A, B and D, E respectively.

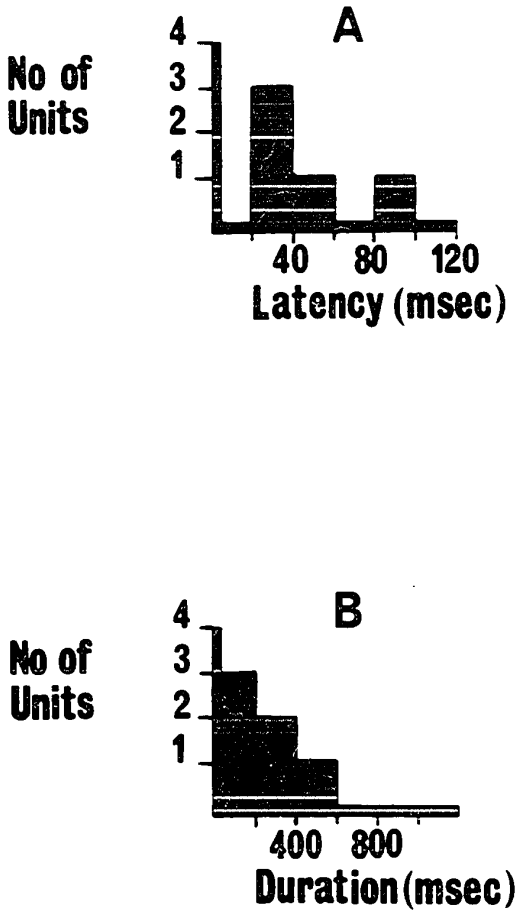

Figure 20. Frequency distribution of latencies (A) and durations (B) of inhibition for a sample of SPNs in the acute spinal preparation inhibited by single shocks to the splanchnic nerve. Ordinate: number of units. Abscissa: latency (msec) in A and duration (msec) in B.

nerve were tested on a population of 86 SPNs. The activity of 31% (18 out of 58) of the SPNs with background activity (either spontaneous or glutamate-evoked) was depressed by single supramaximal shocks to the central cut end of the vagus nerve (Fig. 21). This depression of background activity lasted several hundred milliseconds with single shock stimulation.

In addition to the inhibition of spontaneous or glutamate-evoked discharge, inhibition could also be demonstrated against a background of injury discharges and occasionally of antidromic firing evoked by double shocks applied to the axon of the SPN (Fig. 22). In this instance, with an interval of 5 msec between conditioning and test antidromic shocks, the likelihood of obtaining a response to the test antidromic stimulus was approximately 45% (Fig. 22A). During a period of tetanic stimulation of the vagus nerve at 10 cps and 20 volts the likelihood of obtaining a response to the test stimulus decreased to 17% (Fig. 22B) and then returned to control values (Fig. 22C). In this instance, tetanic stimulation of the vagus did not cause any blood pressure changes.

The threshold intensity for the inhibition was 4V for the one unit in which this measurement was made (approximately 20-25T). Fig. 23 shows a unit, fired by glutamate, which was inhibited at a stimulus intensity of 12 volts (Fig. 23B). When stimulus intensity was increased to 15 volts a reflex excitation appeared with a latency of approximately 190 msec (Fig. 23C). With a further increase in stimulus intensity the excitation was suppressed (Fig. 23D). Thus, this unit shows a) inhibition from vagal afferents can

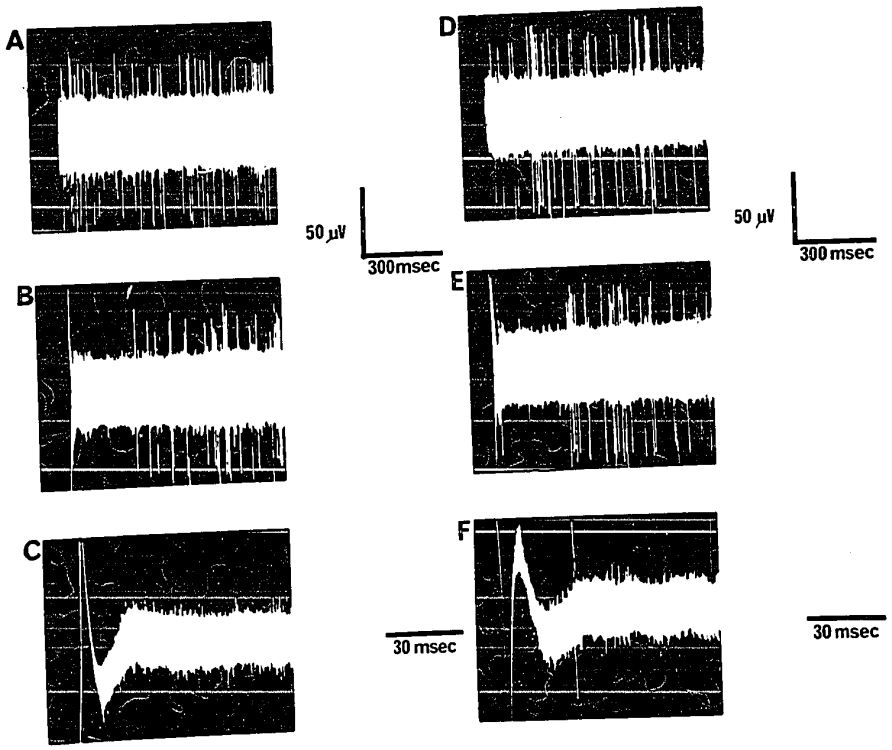

Figure 21. Inhibition of glutamate-evoked activity of SPNs by single shocks to the cervical vagus nerve. A,B,C: data from one SPN and D,E,F from another SPN. A,D: control. Superimposed sweeps (45 in A and 21 in D) triggered by any spike. B,E: shocks to the vagus nerve (15V., 0.5 msec). Superimposed sweeps (45 in B and 21 in D) triggered by the stimulus pulse. C,F: same as in B and E, but at faster sweep speed to show absence of excitation. Uppermost time calibrations apply to A,B and D,E respectively.

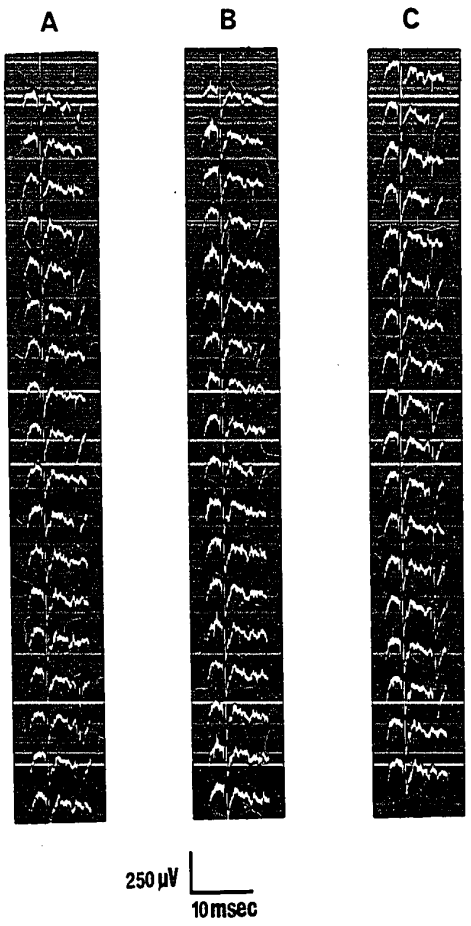

Figure 22. Inhibition of antidromic invasion of stimulation of cervical vagus nerve afferents. Antidromic shock pairs given every 3 sec. Each sweep shows the spike response to the conditioning stimulus (first spike; always present) and the spike response to the test stimulus (second spike; present only at times). In A, before vagal stimulation, the unit responds to the test stimulus 9 out of 18 times. In B, during vagal stimulation at 10/sec, 20 Volts and 0.5 msec pulse duration the unit responds to the test stimulus 3 out of 18 times. In C, after vagal stimulation was terminated, the number of responses to the test stimulus increased to 10 out of 18. There was no blood pressure change during afferent stimulation.

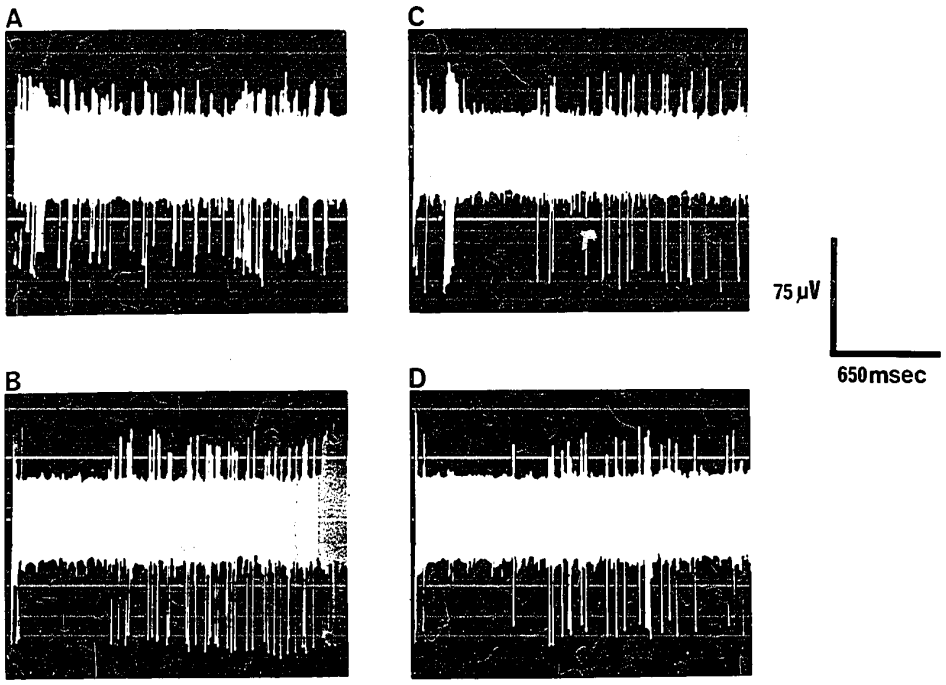

Figure 23. Response of a SPN fired by steady glutamate administration to single shocks of increasing intensity to the cervical vagus nerve. A: control. 20 superimposed sweeps triggered by any spike. B,C,D: shocks to the vagus nerve (12V. in B, 15V. in C, and 25V. in D, 0.5 msec throughout). 20 superimposed sweeps triggered by the stimulus pulse.

appear at a stimulus intensity lower than that required for excitation, b) that an additional inhibition is obtained by stimulating higher threshold afferents and c) the latter inhibition can block the reflex discharge of the SPN evoked by afferents in the same nerve. This inhibition by high threshold vagal afferents is probably the neurophysiological basis for an earlier observation of Douglas and Ritchie (1956) who, while recording systemic arterial pressure and stimulating the cervical vagus, observed that only when the non-medullated afferents of the vagus are stimulated do maximal reflex hypotensive effects occur. These fibres were shown to have a conduction velocity of about 1m/sec.

The latency of the inhibition evoked by the vagus, measured from post-stimulus histograms in 15 units, either spontaneously active or glutamate-evoked, was, on the average, 95 msec (range 30 to 240). No estimates of central transmission time were made. The frequency distribution of latencies in the sample is shown in Fig. 24A. The duration of inhibition with single maximal stimuli, was on the average (19 units) 500 msec (range 180 to 960). The inhibition was of several hundred msec duration even at threshold stimulus intensity. The frequency distribution of durations of inhibition is shown in Fig. 24B.

(d) Inhibition from the Depressor Area of the Medulla

The effects of medullary stimulation (see Methods) were tested on a population of 32 SPNs. Both inhibitory and excitatory effects could be produced. The activity of 8 units (25%) which fired re-

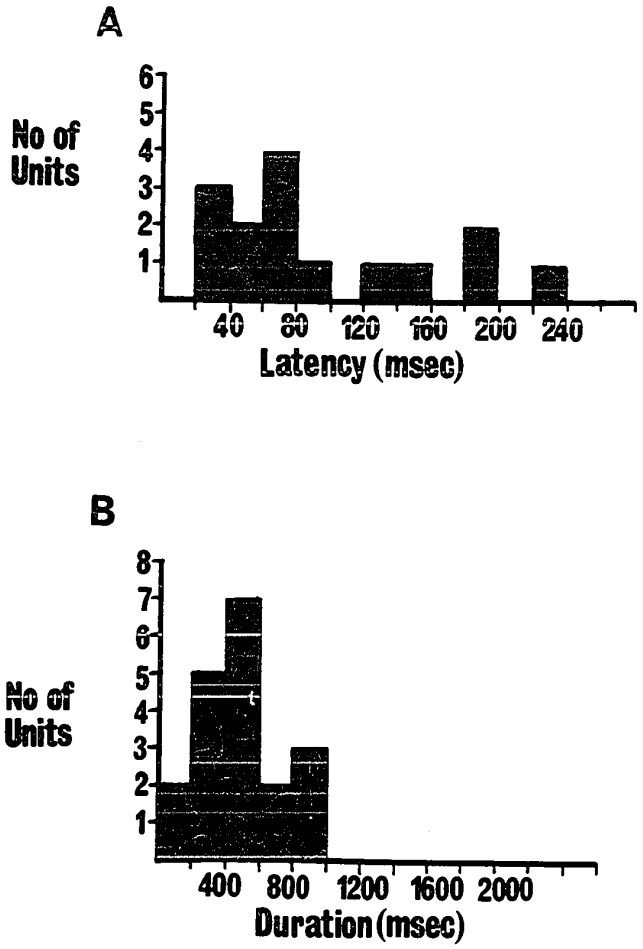

Figure 24. Frequency distribution of latencies (A) and durations (B) of inhibition for a sample of SPNs inhibited by single shocks to the cervical vagus nerve. Ordinate: number of units. Abscissa: latency (msec) in A and duration (msec) in B.

petitively (either spontaneously or as a result of glutamate administration) was depressed by single shocks (Fig. 25). The inhibition could also be demonstrated against a background of injury discharge (2 units). Inhibition of antidromic firing by medullary stimulation was seen in two additional units which were not spontaneously active.

The latency of the inhibition, measured from post-stimulus histograms in 8 units, either spontaneously active or glutamate induced, was, on the average, 54 msec (range 20 to 140). The frequency distribution of latencies in the sample is shown in Fig. 26A. The conduction velocity of the inhibitory volley within the CNS, calculated on the assumption that the volley travelled the shortest distance between stimulating and recording sites was 0.9 m/sec on the average (range 2.5 to 0.4). The duration of the inhibition with single maximal stimuli was, on the average, 430 msec (7 units) (range 250 to 520, Fig. 26B).

(e) Inhibition from the Lateral Funiculus of the Spinal Cord

The effects of stimulation of fibres in the lateral funiculus of the spinal cord were tested on 6 SPNs in the acute spinal preparation and on 6 SPNs in the intact preparation. The activity of 5 SPNs (3 of which were in the spinal preparation and 2 in the intact) with background firing, either spontaneous or glutamate induced, was depressed by single shocks (Fig. 27). The sites of stimulation of the lateral funiculus were at depths 1-2 mm from the dorsal surface

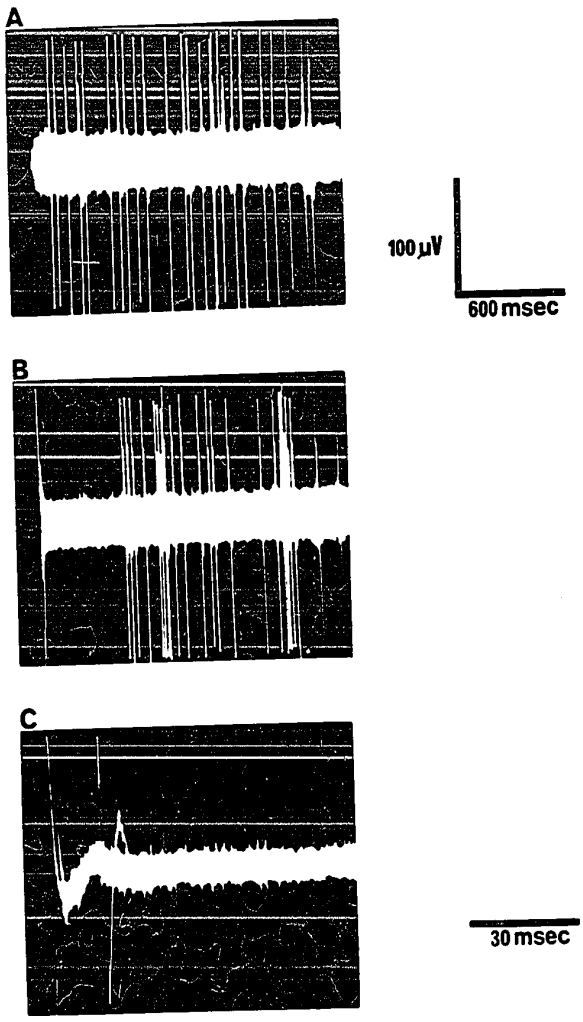

Figure 25. Inhibition of the spontaneous activity of a SPN by single shocks to the depressor area of the medulla oblongata. A: control. 14 superimposed sweeps triggered by any spontaneous spike. B: shocks (3V., 0.3 msec) to the medulla. 14 superimposed sweeps triggered by the stimulus pulse. C: same as in A, but at faster sweep speed to show absence of excitation. Uppermost time calibration applies to A and B.

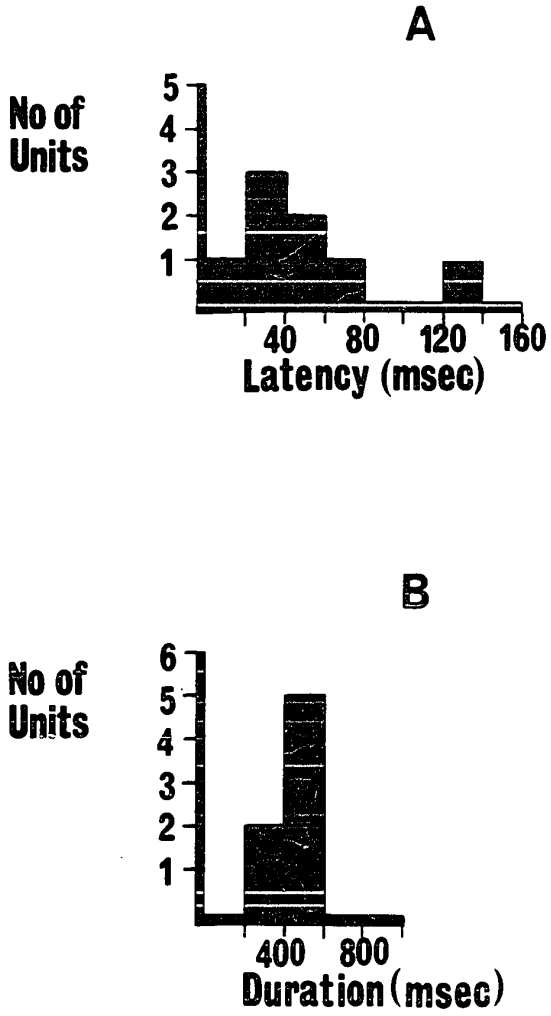

Figure 26. Frequency distribution of latencies (A) and durations (B) of inhibition for a sample of SPNs inhibited by single shocks to the medulla oblongata. Ordinate: number of units. Abscissa: latency (msec) in A and duration (msec) in B.

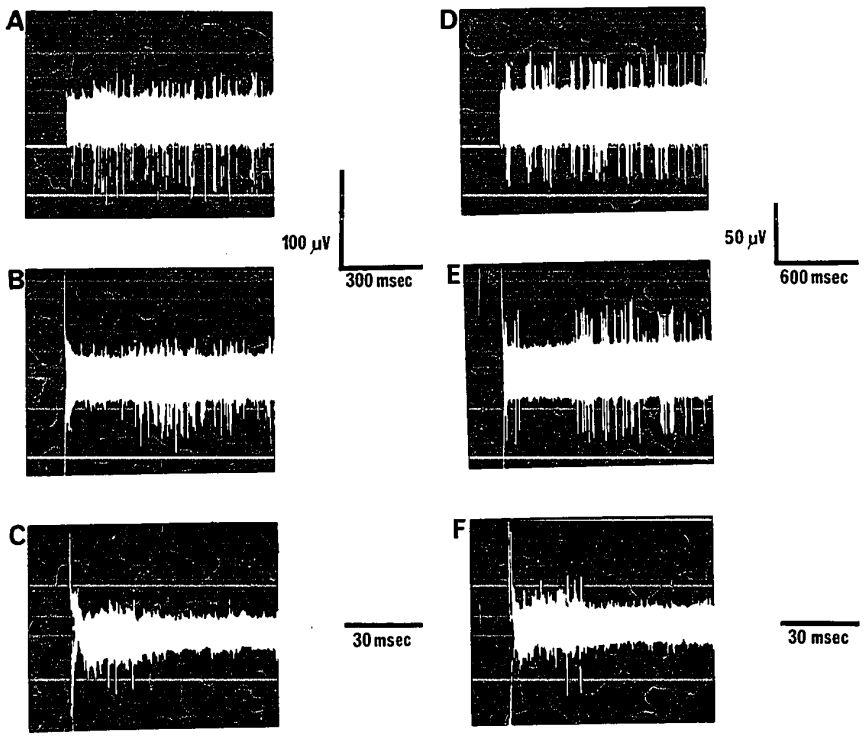

Figure 27. Inhibition of spontaneous (A-C) and glutamate-evoked (D-F) activity of two SPNs in the acute spinal cat. A and D: control. Superimposed sweeps (15 in A and 17 in D) triggered by any spike. B and E: shocks (3V., 0.3 msec) to the lateral funiculus of the spinal cord. Superimposed sweeps (15 in B and 17 in E) triggered by the stimulus pulse. C and F: same as in B and E, but at faster sweep speed to show absence of excitation. Uppermost time calibrations apply to A, B and D, E, respectively.

beneath the entry zone of the dorsal rootlets at the level of the 2nd or 3rd cervical segments. The latency of inhibition, measured from the post-stimulus histograms was, on the average, 39 msec (range 20 to 60). The mean conduction velocity for the inhibitory volley for the three units in the spinal animal was 0.7 m/sec. For the two units in the intact animal, again on the assumption that the volley travelled the shortest distance between stimulating and recording sites, the conduction velocity was 1.9 m/sec. The duration of inhibition with single maximal stimuli was on the average 635 msec (range 160 to 1600).

(iv) Post-excitatory Depression (PED)

It has been pointed out in the introduction, that the scarce neurophysiological data relevant to SPN inhibition consist mostly of records of the electrical activity of whole sympathetic nerves or of bundles containing several nerve fibres. In these recording conditions, what has been considered evidence for inhibition has been a stimulus-locked decrease in the amplitude and frequency of the background electrical waveform (which, in multifibre recording, depends on the number of active fibres and on the patterns and degree of correlation of their firing). Most often the decrease in amplitude of background firing (or silent period) is preceded by an increase, i.e., a reflex excitation (Koizumi et al., 1968, 1971; Iwamura et al., 1969; Jänig and Schmidt, 1970). Another phenomenon,

considered evidence for inhibition, is the depression of a test reflex by a conditioning afferent volley, which always causes some SPN firing (Beacham and Perl, 1964; Franz et al., 1966; Sato et al., 1967). The interpretation of these records was that some units were excited and others inhibited. It would seem that for most of the observations reported, a PED could be considered either as an alternative explanation or as an additional factor that may add to a hypothetical inhibition and distort its time course. Thus, it would seem useful to have an estimate of the time course of the PED. Some authors (Franz et al., 1966) have suggested that PED may be of short duration in SPNs: their arguments were that some SPNs can be re-excited orthodromically at short intervals (but the intensity of the stimulus was not specified) and that the duration of the after-hyperpolarization (AHP), seen in a few cells by intracellular recording, was less than 100 msec (Fernandez de Molina, et al., 1965). However, the first evidence is clearly more relevant to the question of the duration of the absolute refractoriness of SPNs, and the second is from a sample so small that probably no generalization can be made as to the average duration of the AHP for the SPN. Other authors (Polosa, 1967) have shown that the PED of SPNs is long (several seconds) after repetitive firing, but it is impossible to extrapolate from these data to the effect of a single firing, since it is known that for some neurons (Kuno et al., 1970) phenomena of post-excitatory depression are somewhat complex and PED duration is not a linear function of the number of firings. Thus, it was of interest to arrive at

an estimate of the duration of the PED of SPNs after a single action potential. Apart from the reason that this information could help in the interpretation of such data as the reflexly evoked excitation-depression sequences in multifibre preparations, mentioned above, another reason is that some of the spontaneously firing units in the present study presented mixed responses to afferent or CNS stimulation, i.e., excitation followed by a reduction in firing rate. The question was then how to interpret this "secondary" depression: was it due to PED or to true inhibition or to both? Could a criterion based on duration as suggested by Franz et al., (1966), i.e., PED is short, inhibition is long, be used?

The approach adopted here was to study the time course of the post-spike firing probability, for a given unit with background firing (either spontaneous or glutamate-evoked), after background, antidromic and reflex firing. This was done, for some units, by using the autocorrelogram and post-stimulus histogram facilities of the Burns computer. For other units, measurements were made, on a storage oscilloscope face, of the duration of the minimum interval between any background, antidromic or reflex spike, and the earliest following background spike, by superimposing several sweeps triggered by any spontaneous spike or by the antidromic or orthodromic stimulus respectively. Fig. 28 is an example of a spontaneously firing SPN which reponds to medullary stimulation with an early excitation followed by a depression of spontaneous firing. This unit was spontaneously discharging at a mean rate of 0.5 spikes/sec. A single shock to the medulla

evoked a discharge of this unit with a latency of 9 msec (Fig. 28E). The firing index was close to unity and there were occasional double responses separated by an interval of 10-15 msec. Fig. 28A shows 20 superimposed sweeps, triggered by the stimulus to the medulla. Following the evoked discharge, the background firing is depressed for 340 msec (interval between the stimulus-evoked spikes and the earliest spontaneous ones). Fig. 28B shows 20 superimposed sweeps of spontaneous activity, the sweep being triggered by any spontaneous spike. The minimum interval between consecutive spikes is 270 msec (from beginning of sweep to first spike). This low probability of firing, in the wake of a spontaneous spike, has been shown by Mannard (1970) to be due to some property of the SPN (likely a PED) and not of presynaptic neurons generating the background activity. Fig. 28C shows 20 superimposed sweeps, triggered by the antidromic stimulus to the ipsilateral cervical nerve. The minimum interval between antidromic spikes and the earliest following spontaneous ones is 300 msec. Fig. 28D shows twenty sweeps, triggered by a just subthreshold antidromic stimulus. It can be seen that the interval between the subthreshold antidromic shock and the earliest spontaneous spike approaches zero. This fact makes it unlikely that the long silent interval of Fig. 28C is due to recurrent inhibition. The silent intervals of Fig. 28A and 28C are somewhat longer than that of Fig. 28B, probably because some of the orthodromic and antidromic firings occur very shortly after a preceding spontaneous spike and the PED of these two contiguous

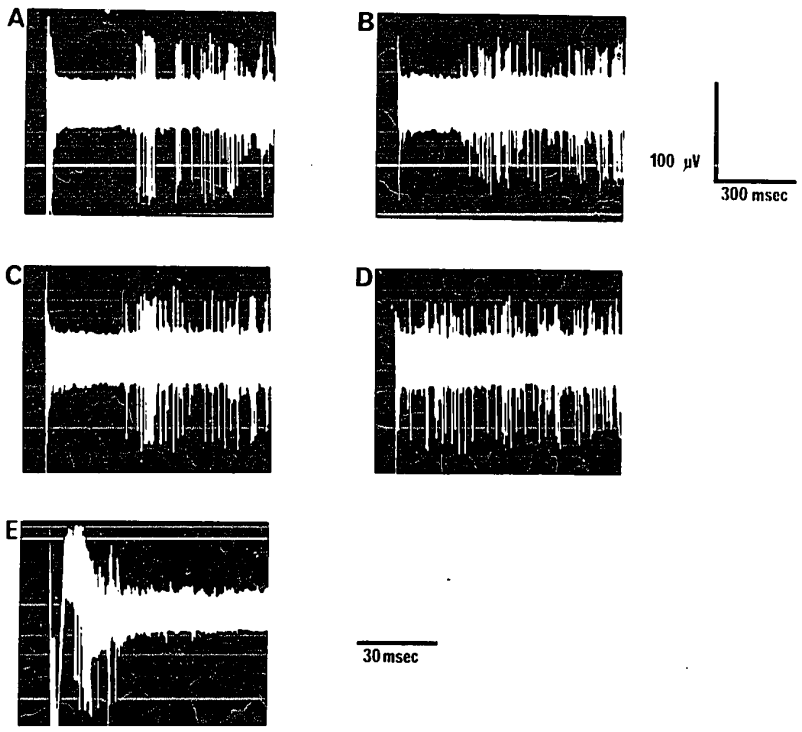

Figure 28. Comparison of duration of the silent period of a SPN following reflex (A and E), antidromic (C) and spontaneous (B) firing. 20 superimposed sweeps in each record. Sweeps are triggered by the stimulus to the depressor area of the medulla oblongata (20V., 0.3 msec) in A and E, by a suprathreshold (20V., 0.3 msec) antidromic stimulus in C, by a subthreshold (8V., 0.3 msec) antidromic stimulus in D and by any spontaneous spike in B. E: as in A but at a faster sweep speed showing the latency distribution of the reflex responses to medullary stimulation. The reflex response had a minimum latency of 9 msec and an average firing index of 1.0 spikes/stimulus. Note that with subthreshold antidromic stimulation (D), the interval between the stimulus and the subsequent spontaneous firing approaches zero. The time calibration next to B applies to A,B,C and D.

firings may summate. Also some of the orthodromic firings were in doublets, so that summation of post-firing effects may have occurred. This experiment shows that for some units the duration of the period of reduced firing probability after an orthodromically evoked, a spontaneous and an antidromic spike can be accounted for in terms of post-firing depression.

Fig. 29 is another example of the same phenomenon. This SPN responded to stimulation of the cervical vagus nerve, with an early excitation followed by a depression of spontaneous firing. This unit was spontaneously active at a mean rate of 1.3 spikes/sec. A single shock to the vagus nerve evoked a discharge of this unit with latency of 120 msec. Firing index was 0.9 spikes/stimulus. Fig. 29A shows 28 superimposed sweeps, triggered by the stimulus to the vagus nerve. Following the evoked discharge, background firing is suppressed for approximately 550 msec (interval between the midpoint of the stimulus evoked spike cluster and the earliest following background spike). Fig. 29B shows the same number of superimposed sweeps of spontaneous activity. The sweeps are triggered by any spontaneous spike. The minimum interval between consecutive spikes is approximately 430 msec (from beginning of sweep to first spike(s)). Fig. 29C shows superimposed sweeps triggered by the antidromic stimulus to the ipsilateral cervical nerve. The minimum interval between the antidromic spikes and the earliest following spontaneous one(s) is approximately 500 msec. When the antidromic

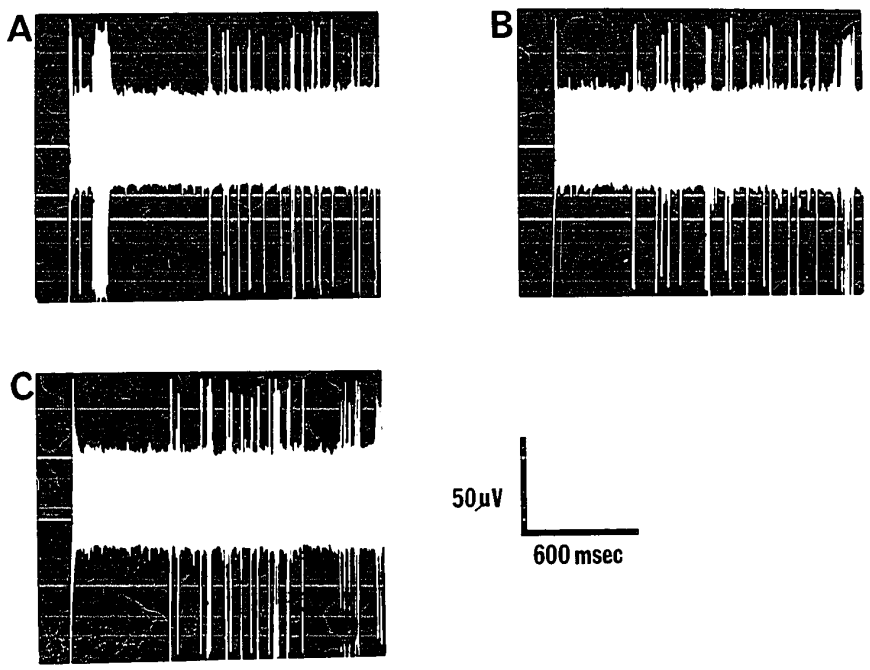

Figure 29. Comparison of duration of the silent period of a SPN following reflex (A), antidromic (C) and spontaneous (B) firing. 28 superimposed sweeps in each record. Sweeps are triggered by the stimulus to the cervical vagus (20V., 0.5 msec) in A, by a suprathreshold (20V., 0.5 msec) antidromic stimulus in C and by any spontaneous spike in B. The reflex response of the unit in A had a minimum latency of 120 msec and an average firing index of 0.9 spikes/stimulus. With subthreshold antidromic stimulation (not shown) the interval between the stimulus and subsequent spontaneous firing approached zero.

stimulus was of intensity just below that which would initiate an impulse of the neuron under study, the intervals between the subthreshold antidromic stimulus and the earliest spontaneous spike again approached zero (not shown). Thus, again, for this unit, the period of lowered post-firing probability was of comparable duration after a reflex, antidromic and spontaneous spike, suggesting that PED is the main determinant of the lowered post-firing probability.

Fig. 30 shows another comparison of duration of silent period following reflex (A), spontaneous (B) and antidromic (C) firing. Fifty superimposed sweeps are triggered by the stimulus to the ulnar nerve in A, by any spontaneous spike in B and by the antidromic stimulus in C. The reflex in A had a minimum latency of 65 msec and an average firing index of 0.8 spikes/stimulus. The minimum intervals were 440 msec in A, 500 in B and 640 in C. With subthreshold antidromic stimuli, the interval between stimulus and subsequent spontaneous firing approached zero.

Fig. 31 shows another unit, for which the silent period following a discharge evoked by stimulation of the medulla (A) was much longer than that following a spontaneous (B) or an antidromic (C) firing. This unit was excited by glutamate and fired at a mean rate of 3.0 spikes/sec. Single shock stimulation of the medulla evoked a discharge with a minimum latency of 8 msec (E) and with an average firing index of 1.0 spikes/stimulus. Each record shows 20 superimposed sweeps, triggered by the stimulus to the medulla in A and E, by any background spike in B, and by the antidromic stimulus

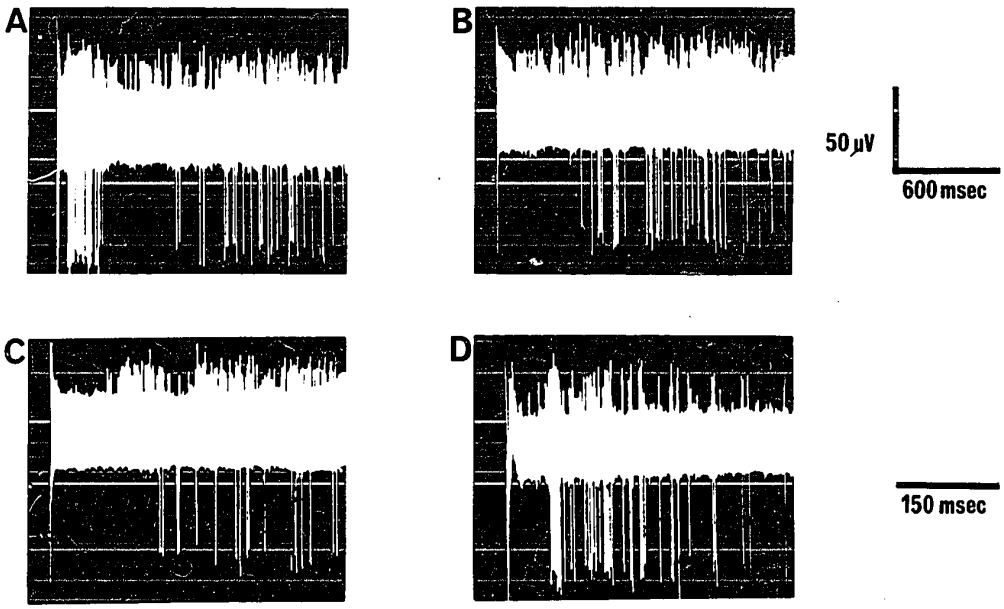

Figure 30. Comparison of duration of the silent period of a SPN following reflex (A and D), antidromic (C) and spontaneous (B) firing. 50 superimposed sweeps in each record. Sweeps are triggered by the stimulus to the ulnar nerve in A and D, by the antidromic stimulus in C and by any spontaneous spike in B. D: as in A but at a faster sweep speed. The reflex response (A and D) had a minimum latency of 65 msec and an average firing index of 0.8 spikes/stimulus. The silent period is longest in C probably because some of the antidromic firing fell close to a preceding spontaneous firing, thus adding to the level of post-firing excitability depression. When the antidromic stimulus was subthreshold for the SPN the interval between stimulus and subsequent spontaneous firing approached zero (not shown). The time calibration next to B applies to A,B and C.

in C and D. The minimum intervals, defined above, were 580 msec in A, 320 msec in B and 300 msec in C. D shows that just subthreshold antidromic stimuli do not evoke a silent period. In the case of this unit, then, the silent period that follows the orthodromic discharge cannot be accounted for entirely by a PED of the SPN, but must be due in part to inhibition, which outlasts the PED by approximately 300 msec.

In summary, from the examples given above one can conclude that the absolute duration of the silent period that follows an orthodromic firing is not an accurate index for deciding whether the silent period is due to inhibition or to PED because PED can be of very long duration in SPNs. Only a comparison of the time course of this silent period and of silent periods which are clearly due to PED gives a reasonable criterion for making this distinction. The above data show that some fairly long silent periods following orthodromic firings could be entirely accounted for by the PED of SPNs (Figs. 28-30), while a silent period of comparable duration (Fig. 31) could not entirely be accounted for in terms of the PED, and therefore could be due to inhibition.

(v) Effects of Strychnine and Picrotoxin on Inhibition of Sympathetic Preganglionic Neurons from Spinal and Supraspinal Inputs

The effects of these two drugs on several types of nervous inhibitions have been extensively investigated and the criterion of sensitivity to these drugs can be used as a basis for a sort of

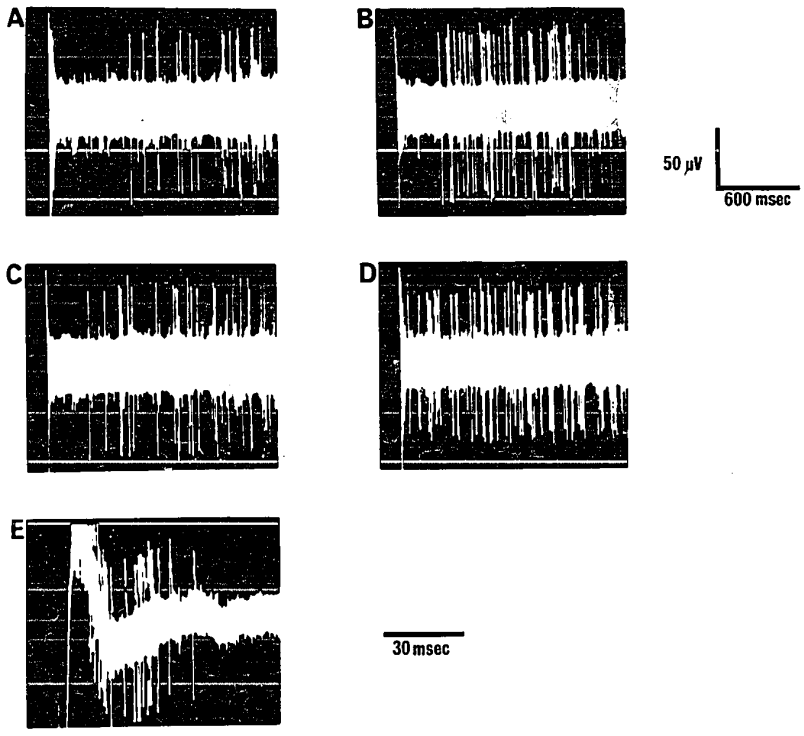

Figure 31. Comparison of duration of the silent period of a SPN following reflex (A and E), antidromic (C) and glutamate-evoked (B) firing. 20 superimposed sweeps in each record. Sweeps are triggered by the stimulus to the depressor area of the medulla oblongata (10V., 0.3 msec) in A and E, by a suprathreshold (30V., 0.3 msec) antidromic stimulus in C, by a subthreshold (10V., 0.3 msec) antidromic stimulus in D and by any spike in B. E: as in A but at a faster sweep speed showing the latency distribution of the reflex responses to medullary stimulation. The reflex response had a minimum latency of 8 msec and an average firing index of 1.0 spikes/stimulus. Note that with subthreshold antidromic stimulation (D), the interval between the stimulus and the subsequent firing approaches zero. The time calibration next to B applies to A,B,C and D.

classification of inhibitory mechanisms. Strychnine has been found to depress several types of postsynaptic inhibitions in the spinal cord, typically the 1A reciprocal inhibition (Bradley et al., 1953) and recurrent inhibition (Eccles et al., 1954). The action of strychnine has been attributed to competitive antagonism with the inhibitory transmitter glycine (Curtis et al., 1967). Picrotoxin, on the other hand, has been found to depress presynaptic inhibition (Eccles et al., 1963). One type of spinal postsynaptic inhibition, however, has been claimed to be picrotoxin-sensitive (Kellerth and Szumski, 1966). It was considered of interest, therefore, to test these drugs on the inhibitory responses of SPNs described so far. Strychnine was administered intravenously to 4 cats in which inhibition of SPN firing could be produced by stimulation of the various inputs. The doses of strychnine ranged from 0.1 to 0.2 mg/Kg. These doses did not significantly alter the mean background firing rate of the tested units nor did they induce strychnine bursts. In all 4 cats, strychnine caused the appearance of an excitation which was not discernible before the administration of the drug. An example of this effect is seen in the post-stimulus histograms in Fig. 32. In this instance the SPN was from a spinal animal and was firing at a mean rate of 1.2 spikes/sec as a result of glutamate release. Each PSH represents 55 sweeps triggered by the stimulus or by an external trigger in the case of the control records. Stimulation of the sciatic nerve (Fig. 32B) produced an inhibition of 72 msec latency (obtained by analysing the record with shorter bin width) and 620 msec duration, ulnar stimulation caused an inhibition of 48 msec

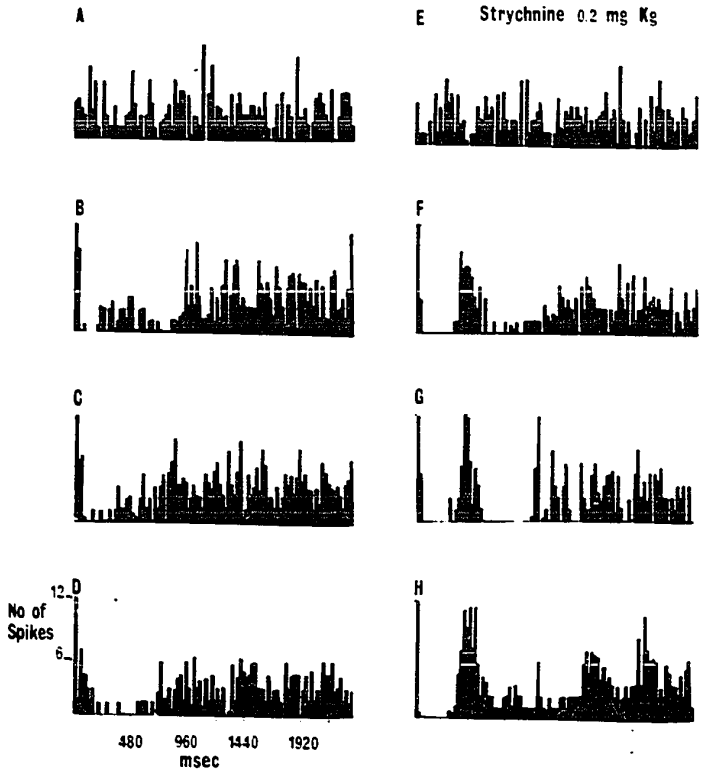

Figure 32. Post-stimulus histograms representing the response of a single SPN, to shocks to the sciatic nerve (B and F), ulnar nerve (C and G), and to the spinal cord at C_2 (D and H), before and after 0.2 mg/Kg i.v. strychnine. Each PSH shows the response of the SPN to 55 shocks. B, C, D: before strychnine, F, G, and H after strychnine. A and E: control background activity before (A) and after (E) strychnine. Calibration in D applies to all records.

latency and 830 msec duration (Fig. 32C) and stimulation of the spinal cord at C-2 caused an inhibition of 96 msec latency and 620 msec duration (Fig. 32D). Strychnine, 0.2 mg/Kg i.v., did not alter the mean firing rate of the cell (compare Fig. 32A and 32E), suggesting that the excitability of the cell was unchanged. Stimulation of the same inputs after strychnine produced, in each instance, an excitation which was not evident previously. This reflex excitation had similar latency (approximately 230 msec) and duration (approximately 250 msec) for all three inputs. Preceding and following this excitation there was a suppression of firing which was no shorter in duration than in the control condition. In fact, the return to control of the cell's mean firing rate after a single stimulus now took longer than before strychnine. The initial period of firing depression preceding the excitation now appeared to be more intense (notice absolute absence of firing; compare Fig. 32F,G,H with 32B,C,D, respectively) than before strychnine.

A similar excitatory effect of strychnine is seen in Fig. 33. The unit in this example was firing at a mean rate of 1.0 spikes/sec and its activity was induced by glutamate. Single shock stimulation of the ulnar and vagus nerves and of the depressor area in the medulla all produced a clear inhibition of the cell's firing (Fig. 33C,D and E). The sciatic nerve also produced a suppression of firing but this depression was preceded by firing which might have been due to a weak excitation (Fig. 33B). After 0.1 mg/Kg strychnine i.v. the mean rate of firing of the cell did not change (Fig. 33, compare A and F).

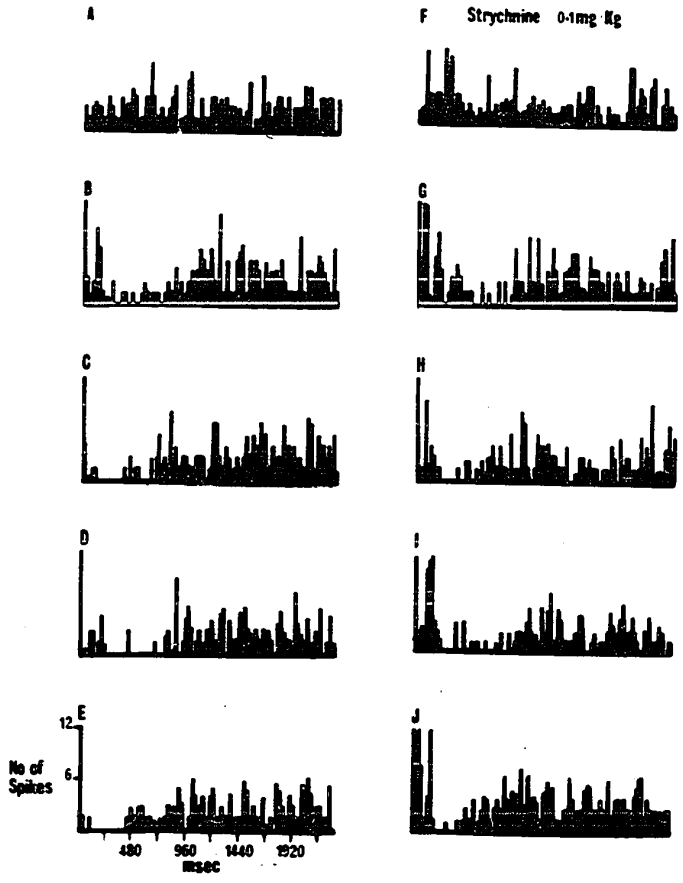

Figure 33. Post-stimulus histograms representing the response of a single SPN to shocks to the sciatic nerve (B and G), ulnar nerve (C and H), vagus nerve (D and I), and to the depressor area of the medulla oblongata (E and J), before and after 0.1 mg/Kg i.v. strychnine. Each PSH shows the response of the SPN to 40 shocks. B,C,D and E: before strychnine, G,H,I and J after strychnine. A and F: control background activity before (A) and after (F) strychnine. Calibration in E applies to all records.

Single shock stimulation of these inputs produced, as for the earlier example, an excitation which was not evident previously. This excitation had a latency of 48, 72, 96 and 48 msec from the sciatic, ulnar, vagus and medullary stimulation sites respectively, and a duration of 48, 24, 72 and 144 msec respectively. A qualitatively similar effect was seen from the two other cells which were tested with strychnine. The appearance, after strychnine, of excitation makes it impossible to draw conclusions concerning the effect of strychnine on the inhibitory pathways because the time course of the inhibition may now have changed on account of the appearance of EPSPs and/or of the superposition of post-firing depression.

Picrotoxin was administered intravenously, in doses ranging from 0.1 to 0.4 mg/Kg, to 5 cats. In 4 cats it reduced the inhibition produced as a result of peripheral nerve stimulation. The latency of inhibition was increased while its duration was decreased. The mean rate of firing of these units was not significantly changed as a result of i.v. picrotoxin. An example of picrotoxin's action is seen in Fig. 34. The SPN in this instance was spontaneously active and firing at a mean rate of 1.5 spikes/sec. Each post-stimulus histogram represents 50 superimposed sweeps triggered by the stimulus artefact or by an external trigger in the case of the control records. Stimulation of the splanchnic nerve (Fig. 34B) produced an inhibition of 48 msec latency and 1300 msec duration. Stimulation after 0.2 mg/Kg picrotoxin resulted in a decreased duration of inhibition (Fig. 34D).

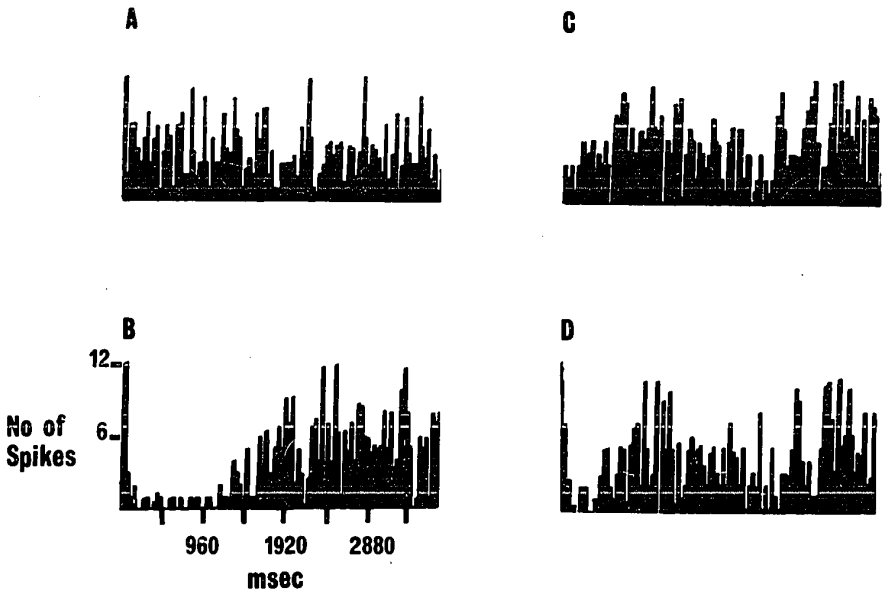

Figure 34. Post-stimulus histograms representing the response of a single SPN to shocks to the splanchnic nerve, before (B) and after (D) 0.2 mg/Kg picrotoxin i.v. Each PSH shows the response of the SPN to 50 shocks. A and C: control background activity before (A) and after (C) picrotoxin. Calibration in B applies to all records.

Similar results can be seen for the unit in Fig. 35. In this case the SPN was firing at 2.5 spikes/sec due to glutamate release. It is seen (Fig. 35C) that after picrotoxin (0.2 mg/Kg) the mean duration of inhibition from the ulnar nerve has decreased considerably. Similar results were obtained with the unit shown in Fig. 36. In this unit, 0.4 mg/Kg picrotoxin reduced the duration of inhibition produced as a result of spinal cord stimulation and increased its latency (Fig. 36C and D).

(vi) Patterns of Convergence of Inhibitory Inputs on Single Sympathetic Preganglionic Neurons

In a group of 74 SPNs in the intact preparation, the effects of stimulation of two or more inputs were tested. The inputs were the sciatic, ulnar, median, vagal and splanchnic nerves, the depressor area of the medulla and descending tracts in the spinal cord. In the spinal animal, two or more of the available inputs were tested on 20 SPNs.

In the intact preparation, 43 SPNs showed convergence, i.e. were affected by at least two inputs. Of these, 16 units were only excited, 14 were only inhibited and 13 were excited by some inputs and inhibited by others. For the spinal animal, 9 units showed convergence. Of these, 7 were only inhibited, one was only excited and one was excited by some inputs and inhibited by others. An example of inhibitory convergence is shown in Fig. 37. This unit was evoked into activity by glutamate and fired at a mean rate of 1.3 spikes/sec. Each record represents an equal number of superimposed sweeps. For this SPN, stimulation of the ulnar (B), sciatic

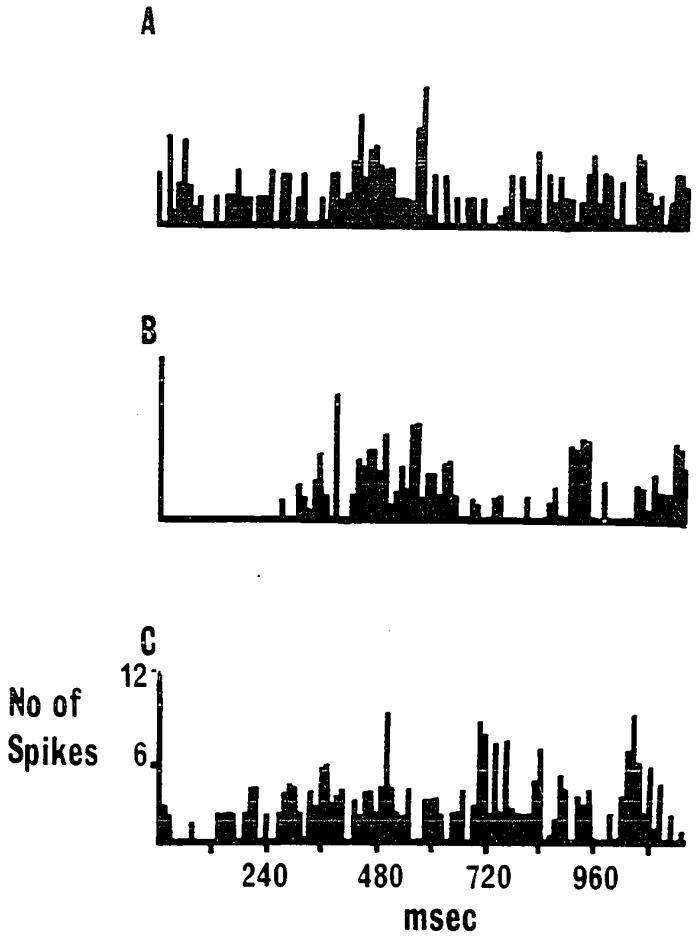

Figure 35. Post-stimulus histograms representing the response of a single SPN to shocks to the ulnar nerve, before (B) and after (C) 0.2 mg/Kg picrotoxin i.v. Each PSH shows the response of the SPN to 50 shocks. A: control background activity before picrotoxin. The drug did not change the mean rate of the cell (not shown). Calibration in C applies to all records.

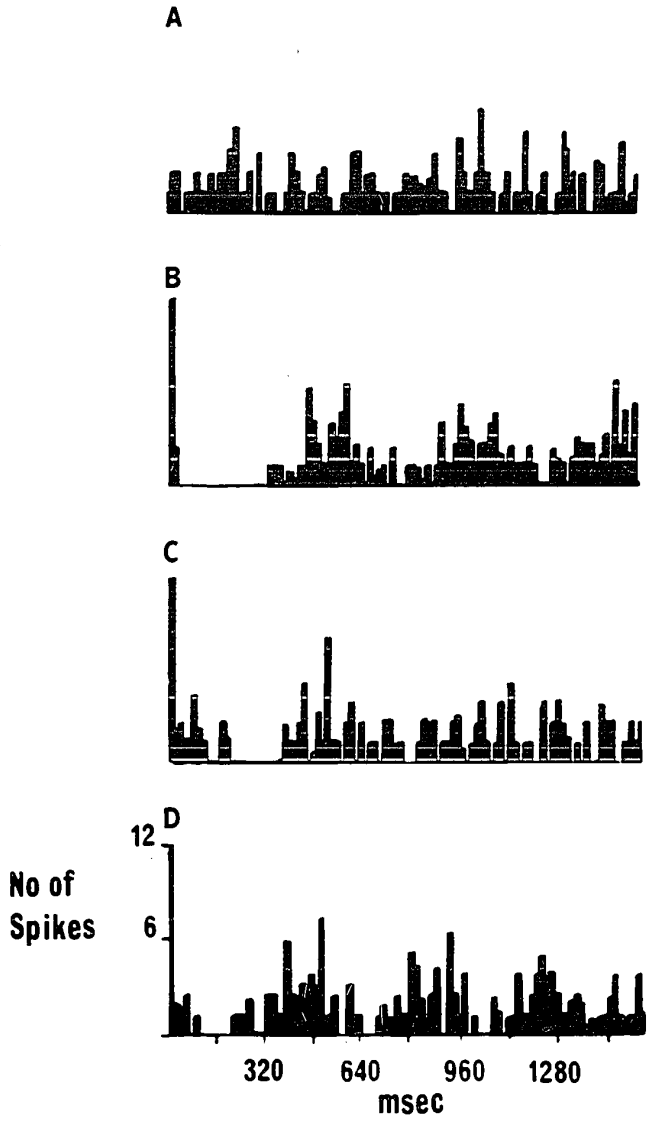

Figure 36. Post-stimulus histograms representing the response of a single SPN to shocks to the spinal cord, before (B) and after (C and D) 0.4 mg/Kg picrotoxin i.v. Each PSH shows the response of the SPN to 35 shocks. A: Control background activity before picrotoxin. The drug did not change the mean firing rate of the cell (not shown). Record in D taken two minutes after record shown in C. Calibration in D applies to all records.

(C) and vagus (E) nerves and the medulla (D) with single shocks resulted in an inhibition of firing which lasted approximately 300 (B), 550 (C), 440 (D) and 350 (E) msec. Analysis at faster sweep speed showed the absence of any early excitation. Thus this cell shows convergence of inhibitory inputs of both spinal and supraspinal origin. Another example of a similar convergence but in the spinal animal is shown in Fig. 38. Single shock electrical stimulation to the sciatic (D), ulnar (E) and splanchnic (F) nerves and stimulation of the spinal cord (C) produces an inhibition of 130 (D), 220 (E), 250 (F) and 260 (C) msec duration.

The inhibitory responses, obtained from the various inputs, in the same unit, or in different units, appeared to have some features in common. One feature is the long duration of the inhibitory effects from all inputs. The duration, in the conditions of these experiments, is influenced by the intensity of the stimulus, and also by the intensity of the background excitation. Very long durations, up to one second or longer, were seen when using strong stimuli and when the background excitation was of low level, e.g. when firing rates, spontaneous or glutamate-evoked, were of the order of 1-2 spikes/sec. However, even when stimulus intensity was low and firing rates were highest (e.g. 15-20 spikes/sec) due to glutamate or injury, the inhibition was of a duration of the order of several hundred milliseconds. Another common feature was that inhibition from any input always was longer lasting than any excitation obtained from the same input, and the lack of excitatory rebound (i.e. post-inhibitory excitation) supports the idea

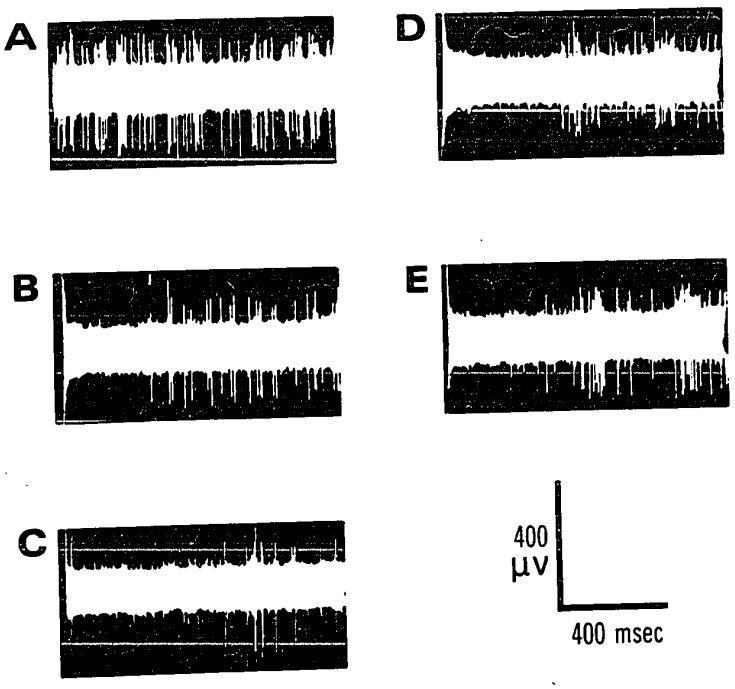

Figure 37. The response of a single SPN to shocks to the ulnar (B), sciatic (C), and cervical vagus (E) nerves and to the medulla (D). 25 superimposed sweeps in each record. A: control background activity. Note the long duration of inhibition (greater than 300 msec) from all the inputs tested.

that the duration of inhibition was long and always outlasted any excitatory effect that might be present. Another feature of interest is that for vagus, splanchnic and limb nerves the inhibition appeared when afferent fibres of similar threshold were excited. Also, picrotoxin reduced the inhibition originating from limb and splanchnic nerve afferents and from descending tracts in the cord. Strychnine, on the other hand, did not seem to interfere with the inhibition but produced complex effects (cf. p.90): these effects were very similar on the various inhibitory responses of a given neuron.

On the basis of these observations, it is possible that the various inputs studied relay to the SPN via a common interneuron. Observations, suggesting the existence of occlusion between inputs, supported the hypothesis of a common inhibitory interneuron. If a common inhibitory interneuron existed, an occlusion of effects might be expected when more than one input was stimulated simultaneously at supramaximal intensity for the observed effect. If all inputs had independent paths to the SPN, a summation of inhibition might be expected. In Fig. 39, taken from a spinal preparation, supramaximal sciatic nerve stimulation is seen to produce an inhibition of glutamate-evoked activity which lasts about 200 msec (Fig. 39B) while descending lateral tract stimulation in the cord results in an inhibition of approximately 350 msec (Fig. 39C). Stimulating both inputs simultaneously with the same parameters produces an inhibition of SPN activity lasting 350 msec (Fig. 39D), i.e. the duration is no longer than that of the inhibition caused by descending tract stimulation alone. This

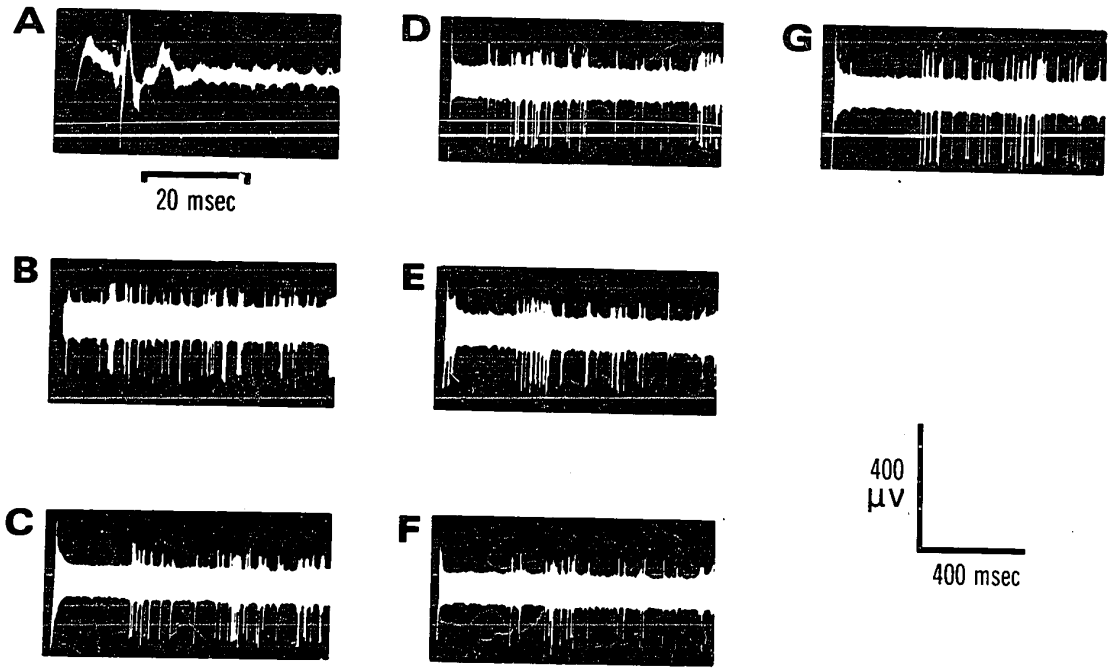

Figure 38. The response of a single SPN, in the acute spinal cat, to shocks to the sciatic (D), ulnar (E) and splanchnic (F) nerves and to descending tracts in the lateral funiculus of the spinal cord (C). 20 superimposed sweeps in each record except for A. A: antidromic identification (6 superimposed sweeps) of the SPN. B: control background activity. G: the response of the SPN to simultaneous shocks to the ulnar and splanchnic nerves.

behaviour could be due to occlusion. However, the possibility exists that summation might have occurred but may have escaped detection due to the unequal duration of the inhibitory effect evoked by each input alone. Since duration of inhibition was the only criterion available in this case for detecting summation, the latter could not have been detected if the time course of the summated effects was no longer than that of the longest lasting individual component.

In another SPN (Fig. 38) supramaximal stimulation of the ulnar nerve (E) caused an inhibition lasting 240 msec, while supramaximal stimulation of the splanchnic nerve caused an inhibition lasting 275 msec (F). Stimulation of both simultaneously (G) evoked an inhibition lasting 320 msec. Since the enhancement of the duration of the inhibition by simultaneous stimulation of both inputs was small, one might suspect that occlusion may have occurred (notice in this case, that the two inputs individually caused inhibition of similar duration). The above tests for occlusion were based on the assumption that with supramaximal stimulation of one input, the hypothetical inhibitory interneuron might be maximally excited and hence may not respond to another input applied simultaneously. Thus the inhibitory response to two inputs supramaximally stimulated may not be larger (or longer lasting) than the response to one of them. If on the other hand, the two inputs used separate interneurons and the convergent point was the SPN, then one would expect summation of the individual IPSPs and one should notice at least a prolongation of the inhibitory duration with respect to that obtained with either input alone.

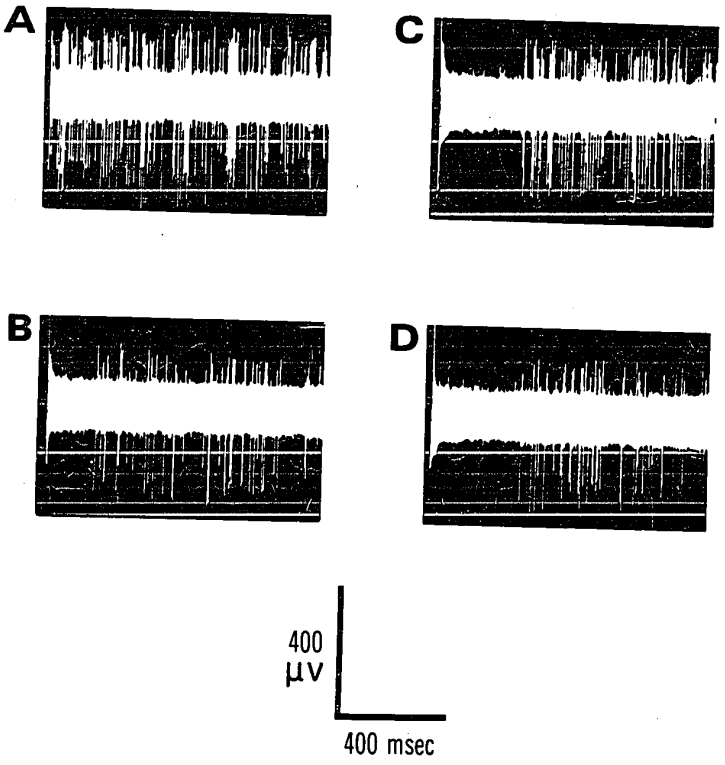

Figure 39. The response of a single SPN, in the acute spinal cat, to shocks to the sciatic nerve (B) and to descending tracts in the lateral funiculus of the spinal cord (C). 28 superimposed sweeps in each record. A: control background activity. B and C: supramaximal shocks to the sciatic nerve and spinal cord respectively, each sweep triggered by the stimulus pulse. D: the response of the SPN to simultaneous shocks to the sciatic nerve and spinal cord.

An alternate test considered as a method for detecting convergence of inputs on a common interneuron was that of spatial facilitation. The test for demonstrating spatial facilitation was performed by adjusting the stimulus intensity of two inhibitory inputs so that it was just subthreshold for inhibition. At this intensity it is postulated that each input produces only subthreshold EPSPs on the hypothetical inhibitory interneuron's membrane. Thus, neither input alone can fire the hypothetical inhibitory interneuron. Now, if the two inputs are stimulated simultaneously (or in the best temporal sequence for achieving simultaneous EPSP generation in the interneuron) and if, as a result, EPSPs are generated on a membrane where they could summate, the EPSP should reach threshold, the interneuron should fire and inhibition of SPN firing should appear. This would be proof of convergence on the interneuron. If convergence were on the SPN membrane, inhibition should not appear (Hultborn and Udo, 1972). Several difficulties were encountered in performing these tests however, mainly because of the time involved in obtaining averages, the large number of trials required for finding the appropriate stimulus intensity which was barely subthreshold for inhibition, and the large variability of neuron responses over time. No conclusive results were obtained.

IV DISCUSSION

In this study, the depression of single sympathetic preganglionic neuron activity evoked by electrical stimulation of spinal inputs (somatic afferents in limb nerves and visceral afferents in the splanchnic nerve) and supraspinal inputs (afferents in the cervical vagus nerve, sites in the lower medulla oblongata and in the lateral funiculi of the cervical spinal cord) has been analyzed. All these sources could suppress various types of SPN firing. This suppression of activity was often seen in the absence of any prior excitation, which shows that inhibition, and not post-excitatory depression, is the underlying phenomenon. These results confirm, as far as somatic afferents in limb nerves are concerned, the conclusions already reached by Wyszogrodski (1970) and Jänig and Schmidt (1970). In addition, they provide the first conclusive demonstration of inhibition of SPNs from all the other inputs studied, for which no single unit studies had been done prior to the present one.

A conclusion which emerged from the experiments in which a comparison was made between the time course of the firing depression which is evoked by synaptic inhibition, and that which follows spontaneous or antidromic firing, was that both were of similar duration. In these experiments it was assumed that the "pause" that follows spontaneous and antidromic firing is due to post-excitatory depression. The reasons for attributing the "pause", following antidromic firing, to PED are the already published observations (Polosa, 1967) that the "pause" is absent when the antidromic stimulus is subthreshold and that, with suprathreshold stimuli, the duration of the "pause" is independent of

stimulus strength. Moreover, intracellular recording from SPNs (Fernandez de Molina et al., 1965) has shown that no recurrent IPSPs are generated by antidromic stimulation. Thus, if there is no experimental evidence that recurrent collateral inhibition, of the type described for spinal somatic motoneurons by Renshaw (1941), is exerted, on a particular SPN, by excitation of adjacent SPNs, this mechanism cannot be invoked as the cause of the "pause", and PED appears a more likely explanation. The reasons for also attributing the "pause", which follows a spontaneous firing, to PED, and not to the timing of the input pulses evoking the background firing, have been given by Mannard (1970), and are essentially based on the observation that antidromic firings, interpolated among the spontaneous firings, do not shorten the pause. If the interpretation of these experiments is correct, the conclusion can be reached that time course is not a criterion for distinguishing an inhibitory process from post-excitatory depression. This conclusion would discount the validity of the reasoning adopted by Beacham and Perl(1964), Franz et al., (1966) and Franz (1966) that in the time course of the recovery of somato- or viscero-sympathetic reflexes the initial phase could be attributed to PED and the late phase to inhibition.

The inhibition of SPNs by descending tracts in the cord, and by limb and splanchnic nerve afferents was still present in the acute spinal animal. Several of these observations were made only a few hours after the section. If the inhibition of SPNs occurs through mechanisms similar to those known to act on other neuronal systems, one could infer

from this finding that the inhibitory synapses, mediating the observed effects, are in the spinal cord. Since primary afferents are known to have excitatory effects only (Eccles, 1969), the inhibitory effects of limb and splanchnic nerve afferents must be mediated by inhibitory interneurons and these must be in the spinal cord. For the inhibitory effect obtained from the descending tracts it cannot be said whether long axons of supraspinally located inhibitory interneurons or descending excitatory axons connecting to spinal inhibitory interneurons are being stimulated. Although inhibition was present in the acute spinal animal, some of its properties, in this preparation, were different from those seen in the animal with intact spinal cord-brain stem connections. For limb afferents, the average duration of the inhibitory effect of a single shock was decreased from 1100 msec to 360 msec in the case of the sciatic nerve, and from 720 msec to 265 msec in the case of the ulnar and median nerve (cf. Results, p. 57). Another finding was that repetitive stimulation was required more often in the spinal than in the intact animal (cf. Results, p. 55). for producing inhibition. Similar results were obtained with stimulation of the splanchnic nerve, for which the duration of inhibition with single shocks was reduced in the spinal animal, from 1070 msec to 290 msec. Due to the small sample of units analyzed (5 units in all), no statement can be made concerning possible changes in the duration of inhibition from spinal cord stimulation. In any case, interpretation of data from this group of experiments would be difficult because, in the absence of more accurate controls of electrode

location (e.g. histological) and/or of a more precise characterization of the properties of the pathway(s) responsible for the observed inhibition, there is no certainty that the same descending system was activated in each experiment. Some changes in latency were also observed in the acute spinal animals, the latency of the inhibitory responses to sciatic and splanchnic nerve stimulation being reduced, while the latency of the inhibitory response to ulnar and median nerve stimulation were unchanged (cf. Results, p.55 and p. 63).

The present data cannot explain the changes observed in these spinal animals. However, some possible explanations may be attempted. Concerning the reduction in duration of the inhibitory effect of a single shock, a simple, hypothetical explanation, could be based on the assumption that: a) at least one, and probably several, interneurons are interposed between the primary afferents and the SPNs, and b) that transmission in this path is dependent on facilitation of interneurons exerted by descending tracts, which are interrupted by the transection (cf. Lundberg, 1966). It is conceivable that removal of this facilitation could result in an effect such as that observed in the spinal animals. This explanation could be tested by trying to find evidence for the postulated descending facilitatory pathway. Evidence would be the finding of points, in the white matter of the cord, whose damage or block (e.g. by cold) would be associated with a reduction in the duration of inhibition, and whose stimulation would be associated with a facilitation of the inhibition. Alternative explanations are of course available. One could be based

on a line of reasoning analogous to that adopted by some authors (e.g. Coote and Downman, 1966) for the disappearance of late (cf. footnote p. 6) somato-sympathetic reflexes in the spinal animal. The decreased duration of inhibition, in the spinal animals, would then be attributed, adopting their reasoning, to the loss of a late inhibitory action of a spino-bulbo-spinal circuit, interrupted by the transection. The present data in intact animals do not provide evidence in favour or against the existence of such long loop inhibitory reflex paths. A careful investigation of the time course of inhibition, based on PSH shape or on intracellular data, would be needed in order to reveal whether two discrete inhibitory pathways, one entirely spinal and the other spino-bulbo-spinal, exist from somatic and visceral spinal afferents to SPNs. Another possible explanation is that the low arterial pressure existing in the spinal animal might, by reducing blood flow through the spinal cord, induce a state of hypoxia, which in turn, may impair reflex transmission (Løyning et al., 1964). This hypothesis is contradicted by the observation that, when all the data from spinal animals are pooled together, no correlation is apparent between the mean blood pressure level and the duration of inhibition. In some of these animals the blood pressure had been artificially raised by the administration of metaraminol. Clearly, more experiments are needed in order to arrive at a satisfactory answer to the question of why inhibition is shorter in the acute spinal animal.

Concerning the decrease in latency of the inhibition evoked by

sciatic and splanchnic nerve stimulation in the spinal animal, as compared with the neuraxially intact, again no factual explanation is available. One possible explanation is that, due to loss of descending facilitatory influences in the spinal animal, with consequent impairment of transmission in interneuron networks, the inhibitory pathways with the lowest safety factor for transmission (i.e. the ones with the longest latency) may stop functioning. Thus the proportion of short to long latency responses would be different in a sample of cells from the spinal as opposed to a sample from the intact animal, and the mean latency for the sample would shift towards a shorter value. A similar effect could result from a loss of hypothetical spino-bulbo-spinal circuits, as mentioned above. Yet another possibility is that the decrease in latency of the inhibition from sciatic and splanchnic nerve stimulation, in the acute spinal animal, is due to the loss, in the spinal state, of EPSPs which were masking, in the intact animal, the earliest part of the IPSPs. The removal of such EPSPs could lead to a change of latency as observed. This explanation would be consistent with the already reported observation (cf. footnote, p. 6) that "late" somato-sympathetic reflexes disappear (or are greatly attenuated) after cervical cord section. Still another possibility is that the spinal section results in the release of the somato-sympathetic inhibitory circuit from a tonic descending inhibition, with consequent facilitation of transmission in this pathway (disinhibition).

Concerning the site of action of the inhibitory mechanism(s) responsible for the depressant effects evoked by the various inputs

studied, two possibilities can be considered. One possibility is that the inhibitory mechanism operates on the membrane of the SPN itself, i.e. is due to post-synaptic inhibition. The other possibility is that the inhibitory mechanism operates on neurons which are presynaptic to, and which provide background excitation to the SPN. In this case, the observed depression of SPN activities would be effected by a process of disfacilitation (Eccles, 1969). The first possibility, i.e. a post synaptic inhibition, seems more likely, because glutamate-evoked discharges and, in fewer cases, antidromic invasion and injury discharges, could be blocked by all the inputs studied. However, a disfacilitation cannot be entirely excluded at present, in the absence of the crucial information concerning the behaviour of the input resistance of the SPN and the properties of the membrane potential changes, if present, associated with the depression. This information can only be obtained by intracellular recording.

The inference, from the depression of glutamate-evoked firing, that the observed inhibitory effects were probably postsynaptic, rests on the assumption that glutamate, released by an extracellular pipette, fires the SPN by a direct action on its soma-dendritic membrane. This assumption is based on arguments presented by Krnjević and Phillis (1963) and Krnjević (1964) in the discussion of the mechanism of the excitatory effect of glutamate on cortical neurons. There are two main arguments: a) spike responses are obtained regularly only when the drug is applied within the gray matter, where the highest concentration of cell somata and dendrites are found; and b) when glutamate is

applied with rectangular current pulses, the latency of the resulting neuron firing is too short to be accounted for by an indirect (i.e. synaptic) mechanism of excitation.

It seems unlikely that the observed suppression of glutamate-evoked firing of SPNs by the various inhibitory inputs was in fact due to the removal of a subthreshold tonic facilitation, (i.e. to a disfacilitation) which fired the cell by summating with the glutamate-evoked depolarization, because often the glutamate-evoked discharge was at rates several times higher (e.g. 5-10 times) than those of any spontaneous discharges observed in these neurons (Polosa, 1968; Mannard, 1970) yet it could be completely suppressed by the inhibitory inputs. This leads to the inference that the cell's firing must have been predominantly due to the glutamate-evoked depolarization and further that removal of a hypothetical background excitatory synaptic activity could not have completely abolished the firing. Moreover, in those cases in which the glutamate-evoked discharge was of high firing rate, the pattern of firing was, unlike the spontaneous one, regular (cf. p.40). Since there are reasons to believe that spontaneous synaptic activity is typically irregular (Mannard, 1970), and yet this irregularity did not show up in the discharge pattern of these cells, the conclusion is again that the depolarization generated by this activity must have been minimal (relative to the amount of glutamate-evoked depolarization). The inhibition of firing evoked by drugs released by extracellular micropipettes has often been taken as a criterion for postsynaptic inhibition (e.g. Krnjević et al., 1966;

Ryall, 1970). Intracellular recording has shown some of these inferences to be correct (e.g. Krnjević and Schwartz, 1968). Conversely, a disfacilitatory process, such as that evoked by cutaneous afferents in sacral parasympathetic neurons, does not depress the amino-acid evoked firing of these neurons, while it depressed reflexly-evoked firing (DeGroat, 1971). It must be mentioned, however, that in these neurons the background synaptic activity could be abolished almost completely by reducing bladder pressure to zero, and as these were the conditions in DeGroat's experiments, these observations may not as strongly support the validity of the hypothesis that depression of glutamate firing reflects postsynaptic inhibition.

The inhibition of injury discharge (cf. p. 45) also suggests that the inhibitory process acts on the membrane of the SPN. This kind of discharge, the characteristics of which have been given on p. 42 is probably due to distortion or actual puncture of the neuron's membrane by the recording electrode (which had relatively large tips in this study). It is unlikely that this discharge is caused indirectly by distortion and damage of presynaptic elements because several of these would have to be affected at the same time in order to achieve the amount of spatial summation which is likely to be required for reaching the firing threshold of the neurons (Mendell and Henneman, 1971). Yet, the spikes of these hypothetical presynaptic axons were not seen by the pipette, which was only recording the spike of an element which could also be fired antidromically from the ipsilateral sympathetic cervical nerve. Thus, this kind of discharge most likely

must be caused by currents generated by the distortion of the SPN membrane, and thus a depression of this discharge must be due to a process acting on the SPN membrane itself.

The block of antidromic invasion, observed in a few silent cells, (p. 48) can also be interpreted as a sign of postsynaptic inhibition. In fact, it is interesting to recall that it was just the absence of block of antidromic invasion of motoneurons by an afferent volley, which however diminished the size of the IA monosynaptic EPSP, that led Frank and Fuortes (1957) to postulate the existence of a type of inhibition different from the classical direct one. From this original observation the concept of presynaptic inhibition successively evolved. In the present series of experiments the effect of the various inhibitory inputs was to increase the duration of the apparent refractory period of the soma, as obtained with double antidromic shocks. Although this effect could be achieved by a process removing a tonic background facilitation, this is not likely to be the cause of the observed effect, because all the units on which antidromic testing was performed were silent, which suggests that any background facilitatory synaptic activity, if present, must have been minimal. Moreover, the duration of the apparent refractory period of the SPN soma-dendritic spike, obtained with double antidromic shocks, was similar in two populations of silent SPNs, one with intact neuraxis, the other with sectioned cord (Polosa, personal communication). The amount of background facilitation in the two populations, however, must have been different, as shown by the difference in mean firing rates

found in active cells in these two types of preparations. This observation suggests that background facilitation in these cells, if present, must have been small enough so as not to affect antidromic invasion appreciably. If this reasoning is correct, then one has to infer that all inhibitions obtained from the various inputs studied were of the postsynaptic type. This conclusion is at variance with previously made inferences that sympathetic inhibition by various afferents is exerted at a supraspinal level (Koizumi et al., 1971) or is presynaptic in nature (Sato and Schmidt, 1966; Franz, 1966; Franz et al., 1966). As mentioned in the Introduction, however, such inferences were made on the basis of not entirely convincing experimental evidence. With intracellular recording then, one would expect to observe IPSPs and, if the inhibition was due to an ionic permeability mechanism, conductance changes. Since dendrites of SPNs are short, e.g. 40-50 microns in length (DeCastro, as cited by Fernandez de Molina et al., 1965), even conductance changes occurring at inhibitory synapses on dendrites should be detected by an intrasomatic microelectrode. Of course, the inhibition could also be caused by an electrogenic, synaptically activated, sodium pump (Pinsker and Kandel, 1969), in which case no conductance changes would be expected.

Postsynaptic inhibition, in the spinal cord, is generally described as strychnine-sensitive (Curtis, 1968) and is presumably mediated by glycine (Werman et al., 1968). Some types of strychnine-resistant, yet presumably postsynaptic, spinal inhibitions have been described, however (Kellerth, 1965; Ryall et al., 1972). One of these

is claimed to be picrotoxin-sensitive (Kellerth and Szumski, 1966). On the basis of the limited number of pharmacological experiments, performed in the present study, it would appear that the types of sympathetic inhibition studied are also strychnine-resistant and picrotoxin-sensitive (Results, p.86). However, this conclusion is from experiments in which the drugs were administered intravenously, and since the inhibitory pathways under study were most likely polysynaptic, there is no way of telling whether the overall drug effect observed was the result of an action on the SPN membrane or at other synapses. Thus, a definitive statement concerning the pharmacological properties of these sympatho-inhibitory mechanisms has to await the results of experiments of microelectrophoretic administration of the drugs to the SPN membrane itself.

One point, related to the tests used for assessing the excitability of SPNs, and which deserves some discussion, is the fact that impairment of antidromic invasion by the various inhibitory inputs was a relatively rare occurrence. This is at variance with what is seen in spinal motoneurons (Eccles, 1957), but is similar to what is seen, for instance, with cortical cells (Krnjević et al., 1966). The explanation for this finding may lie in the safety factor for antidromic invasion, which might be large with SPNs, possibly due to the small size of their somata (roughly one order of magnitude smaller than the spinal motoneurons, (Henry and Calaresu, 1972)). Thus, it is conceivable that even a large decrease in excitability of the soma, as may occur during an IPSP, may not decrease

the safety factor to the point of the soma failing to respond to single antidromic shocks. When antidromic shock pairs were used, however, it can be assumed that the safety factor for soma invasion by the antidromic test spike would be decreased to the point where the process of antidromic soma invasion should become very sensitive to variations in membrane potential of the soma, such as those due to EPSPs and IPSPs. The likely events responsible for lowering the safety factor would be on the one hand a reduction in the current generated by the axonal action potential during its early recovery phase after the conditioning firing, and on the other hand a probable increased conductance of the soma membrane during the early post-spike hyperpolarization (Baldissera and Gustafsson, 1970; Fernandez de Molina et al., 1965). The adequacy of antidromic testing, applied in these conditions, for detecting subthreshold facilitatory influences in SPNs, has been reported previously by Wyszogrodski (1970) who found that stimulation of high threshold limb afferents facilitated antidromic invasion in several silent neurons. In the present work, however, the chance of observing inhibition of antidromic invasion was much lower than that of observing a facilitation, with any of the various inputs tested. Yet, when neuron excitability was tested with glutamate, the chancesof observing facilitation or inhibition were approximately equal. A possible explanation for the rare observation of inhibition of antidromic invasion is that the conditioning antidromic spike is followed by an after-hyperpolarization similar in mechanism (i.e.

due to an increased potassium conductance) and time course to that of the alpha-motoneurons (Baldissera and Gustafsson, 1970 ; but cf. Fernandez de Molina et al., 1965). With the conditioning-testing intervals used in the present experiments (e.g. 6-8 msec) the test antidromic spike would appear at the axon hillock at a time when the soma membrane potential is at or near the peak of the after-hyperpolarization. Assuming at this time the membrane potential to be drifiting towards the potassium equilibrium potential, it is possible that an inhibitory process, based on an ionic mechanism with a similar equilibrium potential, may not add much to the already present hyperpolarization.

V. SUMMARY

The purpose of this work has been to elucidate the nature and mechanism of generation of the depressant effects exerted by several synaptic inputs on sympathetic preganglionic neurons(SPNs). Extracellular microelectrodes were used for recording the activity of SPNs in neuraxially intact or spinal cats. The SPNs were identified by their antidromic responses to stimulation of the sympathetic cervical nerve. Spinal (afferents in limb and splanchnic nerves) and supraspinal (afferents in vagus nerve,sites in the medulla and descending tracts in the spinal cord) inputs were tested.

All inputs tested in the neuraxially intact cat could depress the spontaneous activity of a number of SPNs without prior excitation, i.e. produced inhibition. The spinal inputs and the descending tracts of the cord still evoked inhibition in the acute spinal cat. It is concluded,therefore, that the inhibitory synapses,responsible for these inhibitory effects are in the spinal cord. The characteristics of latency and duration of the inhibition from spinal inputs are,however, modified by the procedure of cord section.

The excitability of spontaneously inactive SPNs was tested by means of electrophoretically released Na glutamate and of antidromic stimulation. Excitability was depressed by the various inputs tested. This suggests that the inhibition could be of the postsynaptic type acting on the SPN membrane itself. Injury discharge could also be inhibited.

From a study of the effects of i.v. strychnine and picrotoxin, it is concluded that the inhibition from the various inputs is strychnine-resistant and picrotoxin-sensitive. From the pharmacology and other evidence it is considered likely that the various inhibitions studied are mediated by an inhibitory interneuron common to several inputs.

Note: The work outlined in this summary and presented in detail in the Results section of this thesis is original.

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