MATURATION OF MYOCARDIAL α_1-ADRENOCEPTOR FUNCTIONS IN THE RAT

by

Arjumand Bano Inayatulla

A thesis submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Master of Science

Department of Pharmacology and Therapeutics

McGill University

Montreal, Quebec, Canada

March 1993

Name Arjumand Bano Inayatullah

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

Pharmacology

SUBJECT TERM

0419

SUBJECT CODE

UMI

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES

American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES

Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences

Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences

Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY

General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

ACKNOWLEDGEMENTS

I would like to extend my profound gratitude to Dr. Daya R. Varma for his understanding, constant help, valuable advice and encouragement.

I would like to thank Dr. Barbara Hales for her constant encouragement.

I would also like to extend my thanks to all the academic and office staff and students of the Department of Pharmacology and Therapeutics for their help on numerous occasions.

My special thanks are due to Ian Guest, Ding-You Li and Dr. Ava Singh for their help and friendship, to Dr. Sylvain Chemtob for help in conducting some of the studies reported here and to Dr. Daniel G. Cyr for the translation of the Abstract into French.

I would like to thank Mr. Alan Forster for his willing assistance.

Finally, I would like to thank my family for their love, support and understanding.

This work was supported by a grant from Quebec Heart and Stroke Foundation to Dr. D.R. Varma.

TABLE OF CONTENTS

Title	Page
ABSTRACT	8
RESUME	9
SECTION 1: INTRODUCTION	10
Adrenoceptors	10
1.1 Historical perspective	10
1.2 Adrenoceptor classification	11
1.2.1 ß-Adrenoceptor classification	11
1.2.2 α-Adrenoceptor classification	12
1.3 Structure of adrenoceptors	14
1.3.1 G-protein coupled receptors	14
1.3.2 Molecular biology of G-protein coupled receptors	14
1.3.3 Cloning of adrenoceptor genes and/or cDNA	15
1.3.4 Molecular biology of α_1-adrenoceptor	16
1.3.4.1 Cloning of α_1-adrenoceptor	16
1.3.4.2 Functional expression of the α_1-adrenoceptor cDNA	16
1.4 Adrenoceptor signal transduction mechanisms	17
1.4.1 Coupling to adenylate cyclase	17
1.4.2 Membrane phospholipid metabolism	17
1.4.3 Calcium translocation	18
1.4.4 Alternate signal transduction mechanism	19
1.5 Localization of the adrenoceptors	19

1.6 Structure-activity relationship of sympathomimetic amines 20

1.7 Structure-function relationship of adrenoceptors 22

1.8 Function of adrenoceptors 22

1.8.1 Myocardium 22

1.8.2 Smooth muscle 24

1.8.3 Brain and nerve terminals 24

1.8.4 Effects on metabolism 25

1.9 Development of innervation to the heart 25

1.10 Modulation of myocardial adrenoceptors 25

1.11 Ontogeny of myocardial adrenoceptors 27

1.12 Objectives of the present study 28

SECTION 2: MATERIALS AND METHODS 30

2.1 Materials 30

2.2 Animals and treatments 30

2.3 Methods 32

2.3.1 Functional studies 32

2.3.2 Radioligand binding studies 34

2.3.3 Measurement of 1,4,5 inositol triphosphate (IP_3) 36

2.4 Statistics 37

SECTION 3: RESULTS 38

3.1 Inotropic effects of sympathomimetic agents 38

3.1.1 Inotropic effects of methoxamine 38

3.1.2 Inotropic effects of isoprenaline 42

3.1.3 A comparison of inotropic effects of methoxamine and isoprenaline 42

3.1.4 Inotropic effects of phenylephrine 48

3.1.5 Inotropic effects of noradrenaline 48

3.2 Inotropic effect of methoxamine at 0.5, 1 and 2 Hz 53

3.3 Effect of adrenoceptor blockers 58

3.3.1 Against methoxamine 58

3.3.2 Against isoprenaline 58

3.4 Influence of different treatments on inotropic activity 63

3.4.1 Influence of 6-hydroxydopamine 63

3.4 2 Influence of dexamethasone 63

3.4.3 Influence of triiodothyronine 63

3.5 Adrenoceptor binding assays 68

3.4.1 α_1-adrenoceptor binding 68

3.4.2 β_1-adrenoceptor binding 68

3.6 Effects on inositol 1,4,5 triphosphate 75

SECTION 4: DISCUSSION 77

4.1 General discussion 77

4.2. Conclusions 86

SECTION 5: REFERENCES 87

INDEX OF TABLES

Table 1.	Age-dependent inotropic activity of methoxamine	40
Table 2.	Inotropic activity of methoxamine relative to calcium	41
Table 3.	Age-dependent inotropic activity of isoprenaline	44
Table 4.	Inotropic activity of isoprenaline relative to calcium	45
Table 5.	Comparison of inotropic activity of methoxamine and isoprenaline	46
Table 6.	Inotropic effects of methoxamine and isoprenaline relative to calcium	47
Table 7.	Inotropic activity of different adrenergic agents	51
Table 8.	Inotropic activity of other agents relative to calcium	52
Table 9.	Inotropic effect of methoxamine at 0.5, 1 and 2 Hz.	57
Table 10.	Influence of 6-hydroxydopamine on effects of methoxamine	64
Table 11.	Influence of 6-hydroxydopamine on effects of isoprenaline	65
Table 12.	Influence of dexamethasone and T_3 on effects of methoxamine	66
Table 13.	Influence of dexamethasone and T_3 on effects of isoprenaline	67
Table 14.	Specific binding of [^{3}H]-prazosin	73
Table 15.	Specific binding of [^{3}H]-dihydroalprenolol	74

INDEX OF FIGURES

Figure 1	Concentration-response curve to methoxamine	39
Figure 2	Concentration-response curves to isoprenaline	43
Figure 3	Concentration-response curves to phenylephrine	49
Figure 4	Concentration-response curves to noradrenaline	50
Figure 5	Concentration-response curves to methoxamine at 0 5 Hz	54
Figure 6	Concentration-response curves to methoxamine at 1 Hz	55
Figure 7	Concentration-response curves to methoxamine at 2 Hz	56
Figure 8	Methoxamine effects in 1-week rats after prazosin and propranolol	59
Figure 9	Methoxamine effects in 10-week rats after prazosin	60
Figure 10	Isoprenaline effects in 1-week rats after propranolol and prazosin	61
Figure 11	Isoprenaline effects in 10-week rats after propranolol	32
Figure 12	[^{3}H]Prazosin binding to cardiac membranes of 1-week rats	69
Figure 13	[^{3}H]Prazosin binding to cardiac membranes of 10-week rats	70
Figure 14	[^{3}H]Dihydroalprenolol binding to cardiac membranes of 1-week rats	71
Figure 15	[^{3}H]Dihydroalprenolol binding to cardiac membranes of 10-week rats	72
Figure 16	Effect of methoxamine on IP_3	76

ABSTRACT

Maximal inotropic response of electrically driven (0.5, 1 or 2 Hz) rat ventricular tissues in vitro to α_1-adrenoceptor agonist, methoxamine, was comparable to that of β_1-adrenoceptor agonist isoprenaline up to 2 weeks of age. The inotropic effect of methoxamine decreased to adult levels at 3 weeks so that in tissues from rats 3 weeks and older, its inotropic effect was significantly less than that of isoprenaline The inotropic effect of isoprenaline was not age-dependent The inotropic effect of methoxamine was antagonized by prazosin and that of isoprenaline by propranolol Treatment of pups at birth with dexamethasone, triiodothyronine or 6-hydroxydopamine did not influence the inotropic effect of methoxamine. Radioligand binding studies using ^{3}[H]-prazosin and ^{3}[H]-dihydroalprenolol as ligands and phentolamine and propranolol as displacers, respectively, revealed no age-dependent differences in myocardial α_1- and β_1-adrenoceptor density or affinity. As well the increase in the second messenger inositol triphosphate (IP_3) after methoxamine was less in neonatal than in adult hearts It is concluded that a greater inotropic effect of α_1-adrenoceptor agonists in neonatal rats may be due to unusual receptor-response coupling, which remains to be identified

RESUME

La réponse inotropique maximale de tissus ventriculaire de rat stimulé électriquement (0 5, 1 ou 2 Hz) in vitro a l'agoniste adrénocepteur-α_1, la méthoxamine, était comparable a celle de l'agoniste de l'adrénocépteur-β_1, l'isoprenaline, jusqu'à l'age de deux semaines L'effet inotropique de la méthoxamine a baissé au niveaux de l'adulte chez les rats de 3 semaines et plus. Cet effet inotropique était significativement moindre que celle de l'isoprenaline L'effet inotropique de l'isoprenaline n'était pas dépendent de l'age La prazosine etait antagonique a l'effet inotropique de la méthoxamine et la propranolol a celui de l'isoprenaline. Le traitement de rat a la naissance soit a la dexaméthasone, triiodothyronine ou a la 6-hydroxydopamine n'a pas influencé l'effet inotropique de la méthoxamine Des études de liaisons de ligand radioactif utilisant la [^{3}H]prazosine et la [^{3}H]dihydroalprenolol comme ligand et la phentolamine et la propranolol comme compétiteur, respectivement, ont révélé aucune dépendance d'age soit dans la densité ou l'affinité des adrénocépteur-α_1 at β_1. De plus l'augmentation du second messager, l'inositol triphosphate (IP_3), après le traitement a la méthoxamine était moins élevé dans le néonatal que dans le coeur adulte Il est conclus qu'un plus grand effet inotropique d'agoniste de l'adrénocepteur-α_1 chez les rats nouveau-né pourrait être le résultat d'une réponse inhabituel dans l'accouplement entre le récepteur et la réponse, qui reste à être identifié.

SECTION 1: INTRODUCTION

Adrenoceptors

1.1 Historical perspective

It was first suggested by Dubois-Reymond (1877) that transmission of nerve impulses may be produced either electrically by action currents or chemically by exciting substances formed at the surface of nerve endings In 1895, Oliver and Schafer (1895) showed that extracts from suprarenal glands had pressor effect. In 1897 the active principle in the suprarenal extract was identified as epinephrine (adrenaline) by Abel (Abel & Crawford, 1897).

In 1905, Elliot (1905) suggested that an adrenaline-like substance may be the neurohumoral transmitter because of the similarity between the effects of adrenaline and stimulation of the sympathetic nerves. He also observed that the effector organs responded to the adrenal hormone, long after the degeneration of sympathetic nerves. Other researchers such as Dixon (1907) proposed that the vagus nerve liberated a muscarine-like substance that acted as a chemical transmitter of its impulses. This theory was not accepted until Loewi (1921) demonstrated the slowing of the frog heart rate in the recipient heart upon contact with the perfusion fluid of the donor heart in which the vagus nerve had been stimulated. He proposed that the slowing of the heart rate was due to the liberation of a chemical substance which he referred to as vagus-substance or vagustoff. In 1926, Loewi and Navratil (1926) presented evidence that vagustoff was acetylcholine. They also demonstrated the release of "acceleranstoff" into the perfusion fluid in summer, when the action of sympathetic fibers in the frog vagus, a mixed nerve, predominated over that of inhibitory fibers. Cannon and Uridil (1921) reported the release of an adrenaline-like substance upon stimulation of sympathetic nerves that increased blood pressure and heart rate. This adrenaline-like substance was later identified as the chemical mediator liberated by sympathetic

nerve impulses at neuroeffector junctions, and named sympathin (Cannon and Rosenbleuth, 1933).

Earlier researchers like Barger and Dale (1910) had noted that the effects of sympathetic nerve stimulation could be reproduced more closely by sympathomimetic primary amines rather than by adrenaline or other secondary amines. Investigators such as Bacq advanced the possibility that demethylated (noradrenaline) might be sympathin. But it was von Euler who, in 1946 found that the neurotransmitter in its highly purified form resembled noradrenaline.

The presence of " receptive substances" in the effector cells was proposed in 1905 by Langley. He also proposed that the response to adrenaline depended on the type of substance present. Nerves whose impulses act through the release of noradrenaline are called the adrenergic nerves and the postjunctional sites acted upon by noradrenaline were initially called adrenoceptive (Dale H.H, 1954) but later they were termed adrenergic receptors or adrenoceptors.

1.2 Adrenoceptor classification

Ahlquist (1948) was the first to suggest the existence of two adrenergic receptor populations. He proposed two subtypes "α" and "ß" based on the basis of relative potency of catecholamines in exerting pharmacological effects. The two receptor subtypes could be either excitatory or inhibitory depending on their ability to recognize and response to different drugs.

1.2.1 ß-Adrenoceptor classification

This classification was generally accepted only after the discovery of ß blockers which were used to confirm Ahlquist's classification and also to characterize the adrenoceptors biochemically. Subsequent work determined the fact that ß-adrenoceptors in different tissues did not have identical pharmacological properties. Based on this heterogeneity of tissue response Lands et al (1967) proposed a subclassification of ß-adrenoceptors into $ß_1$-adrenoceptors, which

mediate the cardiac effects of sympathomimetic drugs and β_2-adrenoceptor which mediate bronchodilator and vasodilator effects. Later work has shown that both the subtypes can be present on the same tissue and the same cell (Minneman et al.,1981).

Recently a third type of ß adrenoceptor has been cloned, the β_3-adrenoceptor, which has a homology of 50.7% with β_1- and 45.5% with β_2-adrenoceptor (Emirone et al, 1989). Of the 11 classical ß-adrenoceptor blockers studied only 2 were reported to have acted as blockers of this receptor, while 2 others acted as agonists. It is suggested to be an atypical ß-adrenoceptors, which are present in adipocytes, liver, ileum and the soleus muscle.

1.2.2 α-Adrenoceptor classification

Anatomical classification

α-Adrenoceptors in different tissues possess different pharmacological properties. Studies with α-adrenoceptor antagonists showed inhibition of noradrenaline effects, but they also showed an increase in the release of noradrenaline during the stimulation of sympathetic nerves (Cubeddu et al.,1974). Two different hypotheses were proposed for this effect of α-adrenoceptor antagonists, one hypothesis suggested that the above mentioned effect could be due to the blockade of postjunctional α-adrenoceptors which prevented receptor-neurotransmitter interaction resulting in an increase of noradrenaline release (Brown and Gillespie, 1957). The other hypothesis suggested that the increased release of noradrenaline was due to inhibition of neuronal or extraneuronal uptake of released noradrenaline unrelated to α-adrenoceptor blockade (Langer, 1970). Further studies made it possible to postulate that this was a prejunctional phenomenon mediated by α-adrenoceptor blockade (Langer et al.,1971, Starke et al., 1971).

Subsequent work showed that certain agonists like clonidine and antagonists like phenoxybenzamine could discriminate between pre- and postjunctional α-adrenoceptors

(Dubocovich and Langer, 1974; Starke et al.,1974) led Langer to propose a subclassification of α-adrenoceptors based on anatomical location into α_1-postjunctional and α_2-prejunctional adrenoceptors.

Functional classification

The presence of postjunctional α_2-adrenoceptors observed by some investigators (Schimmel, 1976; Pettinger 1977) led to the notion that anatomical classification alone could not explain the activity of α-adrenoceptors. Berthelson and Pettinger (1977), then proposed the functional classification of α-adrenoceptors based on the type of function mediated by the receptor rather than the anatomical location of the receptor.

Thus α_1-adrenoceptors were proposed to be those that mediate excitatory responses and α_2-adrenoceptors were those that mediated inhibitory responses. There were instances demonstrating the excitatory effect brought about by α_2-adrenoceptor activation (Drew and Whiting, 1979). Recently there are reports of the inhibitory effect brought about by α_1-adrenoceptor activation (Murphy et al.,1991).

Pharmacological classification

Therefore, neither anatomical location nor functional activity could be reliably used to classify α-adrenoceptors. A new classification based solely upon the relative affinities of highly selective antagonists and to a certain extent agonists to the receptor was then developed and is universally accepted (Nichols and Ruffolo, 1991). For example α-adrenoceptors which are activated by selective agonists such as methoxamine, ciralozine or phenylephrine and blocked by selective antagonists such as prazosin, WB4101, or corynanthine in a competitive manner and in low concentrations, are classified as α_1-adrenoceptors. Whereas α-adrenoceptors activated by α-methylnoradrenaline, UK-14304 or B-HT 920 and blocked by low concentrations of yohimbine,

rauwolscine or idoxan, are classified as α_2-adrenoceptors.

1.3 Structure of adrenoceptors

1.3.1 G-protein coupled receptors

Adrenoceptors (α_1,α_2,β_1,β_2 and β_3) are linked to a large family of closely related proteins - the guanine nucleotide binding proteins or G proteins. Thus they are structurally and functionally related to many hormones and neurotransmitters such as the peptide hormones, eicosanoids, visual light receptor rhodopsin and the muscarinic cholinergic receptor (Dohlman et al., 1987; Lefkowitz and Caron, 1988). G Proteins are bound to the inner face of the plasma membrane. These are heterotrimeric molecules consisting of α, ß and gamma subunits, and their classification is based upon the identity of their α subunit. They have a highly homologous guanine nucleotide binding domain. In the inactive state GDP is bound to the α subunit. Activation of the G protein due to agonist occupancy of the receptor causes the α subunit to bind to GTP which then promotes its dissociation from the "ß-gamma" subunit and activates the membrane bound effector system. It is common to find several receptors activating a single G protein or even one receptor regulating the activity of more than one G protein.

1.3.2 Molecular biology of G-protein coupled receptors

Molecular biology of the G protein-coupled receptors shows that it contains seven sequences of 24 to 28 hydrophobic residues which are connected by hydrophillic sequences, N-linked glycosylated extracellular sites in the amino terminus, and a cytoplasmic carboxyl terminus. These hydrophobic regions are proposed to be the putative transmembrane-spanning domains and are connected intracellularly and extracellularly by a series of hydrophillic loops. It is presumed that these hydrophobic domains may form a ligand binding pocket in the plasma membrane

(Lefkowitz & Caron, 1988).

These G-protein coupled adrenoceptors share a conserved structure and membrane topography, the greatest conservation of the amino acid sequence is within the membrane spanning domains. Homology within a subfamily such as between ß-adrenoceptors, α-adrenoceptors, muscarinic receptors and the opsins is about 40 - 50 %. Homology among different subfamilies for example adrenergic versus cholinergic receptors is about 20 - 30 %. The regions of variation in length in this receptor are the third cytoplasmic loop being the longest in α_2-adrenoceptor, the cytoplasmic carboxyl terminal tail being the longest in the α_1-adrenoceptor and to a lesser extent the amino terminal (Lefkowitz et al .,1989). The second and third cytoplasmic loops contain several serines and threonines, representing potential sites for protein kinase C phosphorylation, and may represent a regulatory mechanism for controlling receptor function. This topography has not yet been proven but is based upon its analogy with findings obtained by high resolution electron diffraction for bacteriorhodopsin (Lefkowitz et al.,1989).

1.3.3 Cloning of adrenoceptor genes and/or cDNAs

Recently, the genes and/or cDNAs for all the subtypes of adrenoceptors have been cloned. The three ß-adrenoceptor subtypes show many similarities; they share a common signal transduction mechanism so that only one or two orders of magnitudes are commonly observed in stimulation of adenylyl cyclase and increase in cAMP accumulation and antagonist selectivity. Though they have many similarities they appear to be products of different genes and are not produced by alternate mRNA splicing or cell specific post-translational modifications (Strader et al., 1987). Repeated exposure to agonists of $ß_1$- and $ß_2$-adrenoceptors display a process of desensitization even in the presence of agonists (Hausdorff et al, 1990), the predominant mechanism for which is presumed to be the loss of cellular receptors. In contrast, $ß_3$-adrenoceptor

unlike the other two is reported to be up-regulated upon chronic exposure to agonists (Thomas et al,1992).

The α-adrenoceptor subtypes do not share a common signal transduction mechanism. α_1-Adrenoceptor stimulates hydrolysis of membrane inositols whereas α_2-adrenoceptor inhibits adenylyl cyclase and thereby decreases accumulation of cAMP. Many drugs exhibit different selectivity for the two receptor subtypes (Fain & Berridge, 1979).

1.3.4 Molecular biology of α_1-adrenoceptor

1.3.4.1 Cloning of α_1-adrenoceptor

The α_1-adrenoceptor is a glycoprotein with a molecular weight of about 80 kDa (Lundberg et al., 1984). It was later purified by Cotecchia et al (1988) from DDT$_1$MF-2 cells by detergent extraction from cell membranes followed by affinity chromatography , wheat germ agarose chromatography and gel permeation HPLC. The purified receptor was then cleaved by cyanogen bromide and amino acid sequence analyzed. This was followed by the construction of a radiolabelled oligonucleotide probe which was then used to probe a genomic library. Hydrophobicity analysis of the receptor revealed a structure similar to the other G-protein coupled receptors.

1.3.4.2 Functional expression of the α_1-adrenoceptor cDNA

The cDNAs encoding the α_1-adrenoceptor was ligated to mammalian vectors under the control of Rous sarcoma virus promoter. The cDNA was then transfected into CCS-7 cells for transient expression. The cells were then harvested. The crude membranes were characterized for α_1-adrenoceptor expression using radiolabelled α_1-adrenoceptor antagonist and phospholipase C activity using radiolabelled phosphorus (Cotecchia et al., 1988).

1.4 Adrenoceptor signal transduction mechanisms

1.4.1 Coupling to adenylyl cyclase

The signalling mechanisms for each of the subtypes of adrenoceptors are distinct. β_1-, β_2-Adrenoceptors stimulate adenyl cyclase. The interaction between the receptor and the enzyme is mediated by a G protein termed G_s. Stimulation of the receptor leads to generation of cyclic adenosine 5'-monophosphate (cAMP), which activates cAMP-dependent protein kinase leading to the phosphorylation of numerous cellular proteins (Lefkowitz et al.,1983; Jakobs et al., 1976). The G_s protein can also act directly to enhance the activation of voltage-sensitive calcium channels and may thus provide an additional means of regulation (Brown and Birnbaumer, 1988). The α_2-adrenoceptors inhibit adenylyl cyclase. The interaction between the receptor and the enzyme is mediated by G protein termed G_i. Stimulation of the receptor decreases the intracellular concentration of cAMP, leading to decreased activation of the cAMP dependent protein kinase. The G_i proteins can also activate potassium conductance and inhibit voltage-sensitive calcium channels (Limbird, 1988).

1.4.2 Membrane phospholipid metabolism

Stimulation of α_1-adrenoceptors activates phospholipase C through an unidentified G protein which leads to the hydrolysis of membrane bound polyphosphoinositides primarily of phosphatidyl inositol 4,5 bisphosphate (PIP_2), resulting in the generation of two second messengers, diacylglycerol and inositol 1,4,5- triphosphate (IP_3) (Michell, 1975). Determination of inositol phosphate accumulation has become relatively easy in the presence of lithium, which inhibits breakdown of inositol monophosphates by myo-inositol phosphatases.

Although it is generally excepted that IP3 is associated with mobilization of intracellular calcium and DAG, which activate protein kinase C leading to phosphorylation of specific

cellular proteins, it is not clear if these are the only two second messengers. Other inositol phosphates have been identified which may act as second messengers. Inositol 1-monophosphate and inositol 1,4 biphosphate exist in cyclic forms and are the direct product of phospholipase C attack on phospholipid precursors (Majerus et al,1986).

1.4.3 Calcium translocation

Many of the cellular effects of α_1-adrenoceptor activation are through increases in intracellular calcium These increases could be caused by release of intracellular calcium and/or influx of extracellular calcium. The best understood mechanism for the increase in intracellular calcium is that IP3 stimulates its release from nonmitochondrial pool which is probably the endoplasmic or sarcoplasmic reticulum by the activation of calcium sensitive protein kinases (Streb et al, 1984). The IP_3 sensitive pool accounts for about 30 - 50 % of the calcium released, the remainder of calcium release is IP3 insensitive and may be from a separate membrane compartment that has calcium pumping characteristics (Thevenod et al., 1989).

The anatomical location of the IP_3 sensitive pools is not certain but studies demonstrated the presence of an IP_3 receptor on the nuclear envelope and parts of the endoplasmic reticulum (Suttapone et al, 1988). The release of calcium depends on the binding of IP_3 to receptors that are linked to calcium channels. Evidence for such a channel comes from membrane vesicles located in the sarcoplasmic reticulum of the aortic smooth muscle, which have been incorporated into planar lipid bilayers (Ehlrich and Watras, 1988). Classical calcium channel blockers do not inhibit calcium release from these channels but cinnarizine and flunarizine can inhibit release (Seiler et al,1987). Potassium channel blocker tetraethylammonium is also capable of blocking release (Shah and Pant, 1988). IP_3 is incapable of releasing calcium in the absence of potassium but other monovalent cations can replace potassium, suggesting that this could be a

potassium-dependent calcium channel.

The influx of extracellular calcium is presumed to be by the activation of calcium channels located in the plasma membrane. There are different types of calcium channels: (a) voltage operated channels; (b) receptor operated channels; (c) second messenger operated channels; and (d) G-protein operated channels. An alternate hypothesis for the influx of extra cellular calcium is that calcium first flows into the endoplasmic reticulum before entering the cytosol. Consistent with the latter hypothesis is the observation that release of calcium from the intracellular pool precedes entry of extracellular calcium (see, Berridge and Irvine, 1989).

1.4.4 Alternate signal transduction mechanism for α_1-adrenoceptors

Though the predominant mechanism for signal transduction by α_1-adrenoceptors is through IP3, there is some evidence that other mechanisms may also be involved. There are reports that in hepatocytes there is increase in inositol phosphates which requires extracellular calcium but there is also an increase in cAMP which is independent of extracellular calcium (Morgan et al,1980). Similar results were reported by Johnson and Minneman (1987) in the rat brain. Another unusual phenomenon is observed in the brain in which tissue α_1-adrenoceptor stimulation potentiates increases in cAMP accumulation caused by activation of other receptors (Johnson and Minneman, 1986). In the heart, activation of α_1-adrenoceptor increases IP3 accumulation (Otani et al, 1988, Scholz et al,1988). But there are reports that α_1-adrenoceptor activation can activate degradation of cAMP along with increases in IP3 (Buxton and Brunton, 1985) Thus there may be multiple signal transduction mechanisms for the activity of α_1-adrenoceptors.

1.5 Localization of adrenoceptors

Both α_1- and β_1-adrenoceptors are integral membrane glycoproteins They are placed strategically in the immediate vicinity of adrenergic nerve terminals in peripheral target organs. This

allows them to be activated during stimulation of the adrenergic nerves. These α_1- and β_1-adrenoceptors can also be present on the nerve terminals. There is evidence that some α_2-and β_2-adrenoceptors are located in postjunctional regions remote from sites of release of . The physiological role of these adrenoceptors is not well understood but they may be preferentially stimulated by circulating catecholamines such as (Hoffman and Lefkowitz, 1990).

1.6 Structure-activity relationship of sympathomimetic agents

The affinity of a drug for its receptor and its intrinsic activity are related to the its chemical structure. This relationship between structure and activity is quite stringent, and minor modifications in its structure such as stereoisomerism can result in major changes in its pharmacological activity. As changes in molecular structure need not alter all actions and effects of the drug completely, it is possible to develop different drugs. An example of this is the development of antagonists of hormones and neurotransmitters by modification of the structure of physiological agonists (Hoffman & Lefkowitz, 1990).

The parent structure of sympathomimetic amines is ß-phenylethylamine. The structure of ß-phenylethylamine consists of a benzene ring and an ethylamine side chain and permits substitutions to be made on the aromatic ring, α- and ß-carbon atoms and the terminal amino group to yield a variety of agents with sympathomimetic activity. Since 3,4-dihydroxybenzene is also known as catechol, sympathomimetic agents with catechol nucleus are also called catecholamines.

The structure of sympathomimetic agents influences their activity. Many of the directly acting sympathomimetic agents activate both α and ß adrenoceptors but the ratio of activity depends on the structure of the agent. Substitutions made in its structure can considerably change the activity of the agent from an almost pure α activity to an almost pure ß activity:

Separation of aromatic ring and amino group: Sympathomimetic activity is greatest when two carbon atoms separate the benzene ring from the amino group.

Substitution on the amino group. Substitutions on the amino ring can alter the spectrum of activity of an agent. For example addition of a methyl group to noradrenaline converts it to adrenaline and also increases the ß adrenoceptor activity. In general selective ß-adrenoceptor activity requires a large amino substitution and the less the substitution, the greater the α-adrenoceptor activity.

Substitution on the aromatic nucleus: Maximal α- and ß-adrenoceptor activity depends on the presence of OH group in the 3 and 4 positions. Absence of these groups without any other aromatic substitution can reduce the overall potency. Substitutions of the polar groups on the phenylethylamine structure makes the agent less lipophilic, thus unsubstituted or alkyl substituted agents cross the blood-brain barrier more easily, for example ephedrine and amphetamine. Phenylethylamines that lack both hydroxy groups on the ring and ß hydroxy group on the side chain act by causing the release of noradrenaline from adrenergic nerve terminals and are classified as indirectly acting.

Substitution on the α-carbon atom: This substitution, for example in amphetamine, blocks oxidation by monoamine oxidase, thus prolonging their duration of action. Compounds with α-methyl substitution can persist in the nerve terminal and are more likely to release noradrenaline from sites of storage.

Substitution on the ß-carbon ring: Substitution of the hydroxy group on this ring makes the compound less lipophilic and thereby decreasing its activity in the CNS. However, this substitution greatly enhances α and ß activity e g ephedrine which is less potent in the CNS but more potent in the periphery.

Absence of the benzene ring: The substitution of the benzene ring with a saturated ring or by a different unsaturated ring, for example propylhexedrine, reduces CNS activity without decreasing α and ß activity.

1.7 Structure-function relationship of adrenoceptors

To understand the structure-function relationship of these receptors different approaches have been taken. Techniques such as the creation of deletion mutants in which one or another segment of the receptor gene has been deleted (Dixon et al., 1987), site directed mutagenesis in which the codons for individual amino acids or stretches of amino acids of the receptor gene are altered (O'Dowd et al., 1988, Strader et al 1987), and construction and expression of chimeric receptor genes, have all increased our understanding of structure-function relationships of these receptors (Lefkowitz et al., 1989). The information obtained from the construction and expression of a chimeric α_2-$ß_2$-adrenoceptor suggests that the membrane spanning regions appear to be important for ligand binding and the third cytoplasmic loop appears to be important for the interaction between the receptor and the appropriate G protein (Lefkowitz et al., 1989).

1.8 Function of adrenoceptors

1.8.1 Myocardium

Inotropic effect

The inotropic effect is produced predominantly through the activation of $ß_1$-adrenoceptors (Dukes and Vaugham Williams, 1984; Lands et al, 1967). Both $ß_1$- and $ß_2$-adrenoceptors are reported to coexist in many tissues. Radioligand binding studies have also demonstrated the presence of $ß_2$- along with $ß_1$-adrenoceptors in the hearts of many species (Ablad et al, 1974, Brodde, 1989). Only at very high concentrations is a small $ß_2$-adrenoceptor component

detected, thus β_2-adrenoceptors can mediate inotropic effect but to a lesser extent when compared to β_1-adrenoceptors (Wilson and Lincoln, 1984).

α_1-Adrenoceptors were first reported to mediate positive inotropic effect in the heart in 1966 by two groups (Govier et al ,1966, Wenzel and Su, 1966) Unlike the β-adrenoceptors, radioligand studies found α_1- but not α_2-adrenoceptors in the heart of many species (Corr et al , 1981; Ferry and Kaumann, 1987)

α_1-Adrenoceptor mediated inotropic effect differs from β_1-adrenoceptor mediated effect in many aspects. α_1-Adrenoceptor mediated inotropic effect develops relatively slowly, results in increase of the slow inward current, is accompanied by prolongation of contraction, and is dependent upon the frequency of stimulation, and on temperature (Kunos and Nickerson, 1977, Ledda et al., 1975, Scholz et al.,1986)

Chronotropic effect

As with the inotropic effect, chronotropic effect is predominantly through the activation of β_1-adrenoceptors. β_2-Adrenoceptors are thought to be more abundant in the atria than the ventricles (Baker et al., 1980). However, the physiological role of the myocardial β_2-adrenoceptors is not completely understood, although they have been reported to produce positive chronotropic effects (Yabuchi, 1977). Stimulation of α-adrenoceptors can also produce chronotropic effects Nakashima and Hagino (1981) have reported positive chronotropic effects in the rat atria, whereas Shah et al (1988) have reported negative chronotropic effects following α-adrenoceptor stimulation in the canine purkinje fibers

Arrhythmogenic effect

Both α- and β- adrenoceptor agonists are known to produce arrhythmias Rosen et al , (1977) have reported that the development of automaticity following exposure to catecholamines

is more frequent in the neonatal than in adult hearts.

1.8.2 Smooth muscle

In general, stimulation of α-adrenoceptors causes contraction of all types of smooth muscles including the vascular smooth muscles but relaxation of muscles of the gastrointestinal tract. This relaxation is thought to be due to the stimulation of presynaptic α_2-adrenoceptors. Stimulation of ß-adrenoceptors causes relaxation of most types of muscles.

1.8.3 Skeletal muscle

α-Adrenoceptor stimulation cause an increase in the release of the neurotransmitter. This increase could be due to enhanced calcium influx (Snider and Gerald, 1982). $ß_2$-Adrenoceptor agonists can produce tremors probably due to an increase in the muscle spindle discharge, coupled with an effect on the contraction kinetics of the fibers.

1.8.4 Brain and nerve terminals

α_1-Adrenoceptors are abundant in the brain. Though their precise function is not clear, they may play an important role. Specific agonists and antagonists have marked effects in the membrane currents in neurons (Aghajanian, 1985) and on animal behavior (Agrawal et al., 1984). Presynaptic α_2-adrenoceptors play an inhibitory role and are present on both the adrenergic and cholinergic nerve terminals. They inhibit the release of excitatory transmitters.

1.8.5 Effects on metabolism

Adrenoceptor agonists promote glycogenolysis and mobilize glucose in response to hypoglycemia. This is thought to be predominantly due to the stimulation of β_2-adrenoceptors but α-adrenoceptor agonists can also produce glycogenolysis. ß-Adrenoceptor agonists can produce lipolysis, which is thought to be due to the activation of hormone-sensitive lipase (Hoffman and Lefkowitz, 1990).

1.9 Development of innervation to the heart

Mammalian heart develops from a tubular connection between extraembryonic and intraembryonic blood vessels. The heart is richly innervated by the autonomic nervous system. Sympathetic innervation is from the cardiac branches of the plexus formed on the common carotid artery by nerve fibers from the superior cervical ganglion and/or the cervical sympathetic trunk. Parasympathetic innervation is by the cardiac branch of the vagus The parasympathetic innervation is mainly to the S-A node and to the atria, whereas the sympathetic nerves innervate S-A node, atria and the ventricles (Burnstock, 1969). In studies on the rat, innervation to different areas of the heart develops at different periods of growth (Gomez, 1958). Sympathetic and vagal fibers have been observed at the base of the heart in the 12 day old rat embryo, innervation to right atrium, sinus venosus and the aortic arch on day 14, innervation to S-A node and A-V node on days 15 and 16. The density of innervation increases from the day after birth and the innervation pattern approaches that of the adult animals on day 22 (Schiebler and Heene, 1968).

1.10 Modulation of myocardial adrenoceptors

The proportion of α_1- and β_1-myocardial adrenoceptors differs in different species (Mukherji et al, 1983; Wagner and Brodde, 1978). This proportion can be altered by different drugs

and treatments. Administration of propranolol in rats is reported to increase myocardial α_1-adrenoceptor density without a change in receptor affinity, while the density and affinity of myocardial β_1-adrenoceptors remains unaffected (Mugge et al, 1985). The increase in α_1-adrenoceptor density can be noticed as early as 2 days of treatment with propranolol and can be reversed within 2 days after cessation of treatment (Steinkraus et al, 1989). On the other hand, long term administration of phenylephrine in rats decreases both α_1- and β_1-adrenoceptor density without affecting their respective affinities (Gengo et al, 1988).

Treatments such as application of clips to the renal arteries of rats can induce hypertension, which reduces α_1-adrenoceptor density (Hanna and Khairallah, 1986). Chemical sympathectomy produced by the injection of 6-hydroxydopamine increases α_1- and β_1-adrenoceptor density in rats (Yamada et al., 1980 a,b). Diet can also alter the proportion of adrenoceptors e.g. increased salt uptake induces hypertension which leads to a decrease in β_1-adrenoceptor density but does not affect α_1-adrenoceptor density (Tsuji et al., 1987).

Various metabolic diseases such as diabetes, hypertension, hyper- or hypo- thyroid and pituitary functions, and other factors such as species, temperature and frequency of stimulation can influence the relative importance of α_1- and β_1-adrenoceptors in the inotropic effects of sympathomimetic agents. Injection of streptozotosin induces acute diabetes. In rats, this experimental diabetes is reported to decrease α_1-adrenoceptor density but increases affinity and consequently causes an increased responsiveness to methoxamine (Wald et al., 1987). In spontaneous hypertension there is a reduction of both α_1- and β_1-adrenoceptor density during development of hypertension along with decreased responsiveness to isoprenaline (Yamada et al., 1984).

In euthyroid and hyperthyroid animals inotropic adrenoceptors are predominantly β, where as in the hypothyroid animal there are significantly less β- and more α-adrenoceptors and

α-adrenoceptors contribute significantly to the inotropic effect of sympathomimetic agents (Ishac et al., 1983). Hypophysectomized rats show a decrease in ß response and an increase in α response similar to the changes seen in hypothyroid rats (Kunos et al., 1980) Species dependent difference in the balance of α- and ß-adrenoceptors is suggested by the fact that adrenergic response pattern in the euthyroid rabbit is similar to that of the hypothyroid rat (Kunos et al., 1978). The inotropic response mediated through α_1-adrenoceptors are reported to be significantly higher at 17°C than at 31°C (Kunos and Nickerson, 1977), and at stimulation frequency of 1 Hz than at 2.5 Hz (Ledda et al., 1975).

1.11 Ontogeny of myocardial adrenoceptors

It has been proposed that during the development of autonomic neuromuscular relationships, the postsynaptic component such as the receptors and the membrane conductance mechanisms develop before the presynaptic component (Mackenzie and Staden, 1976; Pappano,1977). Adrenoceptors are reported to appear on day 10 in rat embryos concomitant with the onset of heart function and before the appearance of adrenergic nerves (Robkin et al.,1974).

Most investigators found a higher adrenoceptor density in the neonate when compared to the adult heart. Roeske et al (1981) have reported a significantly higher density of $ß_1$-adrenoceptor density in the neonatal than in adult mouse heart. A similar high α_1-adrenoceptor density in neonatal heart is reported by Yamada et al. (1980). Ishii et al (1982) have reported a developmental decrease in sensitivity to noradrenaline in the atria but not ventricle of the rat. However, chemical and immunological sympathectomy produced a postjunctional supersensitivity in both tissues. Other investigators have reported that administration of triiodothyronine in neonatal rats produce enhanced vesicular uptake of noradrenaline, increased ß-adrenoceptor binding along with supersensitivity of the chronotropic response to isoprenaline (Lau and Slotkin, 1980). The

same investigators have also reported that injection of dexamethasone in neonatal rats did not alter the vesicular uptake of noradrenaline, nor the ß-adrenoceptor density, but altered the chronotropic responses to isoprenaline (Lau and Slotkin, 1981).

1.12 Objectives of the present study

In the adult mammalian heart inotropic responses to sympathomimetic agents are contributed predominantly by the activation of $ß_1$-adrenoceptors (Dukes and Vaughan Williams, 1984; Endoh and Blinks, 1988; Lands et al.,1967) though there is a small but definite contribution by the α_1-adrenoceptors (Benfey and Varma,1967; Govier, 1966; Wenzel and Su, 1966). In a recent study from our laboratory (Varma, 1991), it was found that an α_1-adrenoceptor agonist potentiated the $ß_1$-adrenoceptor mediated inotropic effect on atria of young but not adult rabbits, possibly because of some functional link between the two receptors during development. Other investigators have also reported that the cardiac response to many drugs including neurotransmitters changes with age (Adolph,1971, Freidman,1972).

The work of this thesis is oriented towards characterizing ontogenic differences in the inotropic response of the rat myocardium using methoxamine as α_1- and isoprenaline as $ß_1$-adrenoceptor agonists and finding out the underlying mechanism.

On the basis of preliminary work, the following working hypothesis was formulated:

A greater inotropic effect of α_1-adrenoceptor agonists in neonatal than in adult rat myocardium is due to a higher density in myocardial α_1-adrenoceptors and/or a more efficient receptor-second messenger coupling in neonatal hearts.

To test the above hypothesis, inotropic effect of methoxamine and isoprenaline was

determined on ventricular preparations from 0.5 to 10 week old rats. Attempts were made to modify adrenoceptor functions by using triiodothyronine, dexamethasone and 6-hydroxydopamine. Both α_1- and β_1-adrenoceptor concentrations and effects of methoxamine second messenger, IP_3, were measured in hearts from selected age groups of rats.

SECTION 2: MATERIALS AND METHODS

2.1 Materials

D-Myo-inositol 1,4,5-triphosphate-^{3}[H] assay system was purchased from Amersham, Mississauga, Ont. ^{3}[H]-Dihydroalprenolol HCl (107 Ci/ mmol) and ^{3}[H]-prazosin HCl (76.2 Ci/ mmol) were purchased from Dupont NEN, Mississuaga, Ontario. Dexamethasone, 6-hydroxydopamine, isoprenaline bitartrate, lidocaine, methoxamine, noradrenaline bitartrate, phenylephrine HCl, prazosin HCl, propranolol HCl and triiodothyronine were all purchased from Sigma Chemical Co, St. Louis, MO. All other high purity chemicals were purchased from Fisher or BDH, Montreal, Quebec.

2.2 Animals and treatments

The protocol for the use of animals was approved by the McGill University Animal Care Committee. Adult sprague-Dawley male and female rats were purchased from Charles River, St. Constant, Quebec. Rats were maintained in environmentally controlled rooms at the McIntyre Animal Resource Center at 22-25°C, 50-70% humidity and a 12 hour light-dark schedule (lights on 0700-1900 h). Rats were housed in plastic cages with wood chip bedding. Purina rat chow and tap water was available *ad libitum*. The maximum number of animals per cage was limited to four.

Pregnant rats:

Virgin female rats weighing between 200 to 225 g were mated overnight with male rats weighing 250 to 275 g. Smears were prepared the next morning from vaginal washing and examined under microscope. The presence of sperm in the vaginal wash designated day zero of pregnancy. Pregnant rats were housed individually approximately 1 week before delivery under the above mentioned conditions.

Selection of rat pups:

The usual litter consisted of 13 - 15 rat pups. Male pups were selected by identifying the external genitalia and were confirmed by identifying the testes after dissection. Some male pups, picked from different litters were used to study the responses in the same age group. Other male pups were allowed to grow and used for the study at different ages.

6-Hydroxydopamine (6-OHDA) treatment:

6-OHDA was freshly dissolved in 0.9 N saline and 0.1% ascorbic acid. Rat pups were injected i.p. with 150 mg/kg 6-OHDA starting on the first day after birth and then daily for 3 days. Some pups were studied on day 7 and others were allowed to grow to be 3- and 6-weeks old The 3-week old rats were injected once more at 2 weeks and 6-week old rats were injected at 2 weeks and at 4 weeks with 150 mg/kg 6-OHDA.

Dexamethasone treatment:

Rat pups were injected i.p. with 1mg/kg dexamethasone dissolved in 0.9 N saline starting on the same day as birth, and thereafter injected daily for 3 days; pups were studied at the age of 1 week. Adult rats were also injected with dexamethasone for 3 days and studied 3-4 days after stopping the treatment.

Triiodothyronine treatment:

Rat pups were injected i.p. with 0.1 mg/kg triiodothyronine dissolved in 0.9 N saline solution starting on the same day as birth and thereafter injected daily for 6 days and were studied 24 hours after the last injection (7 days old).

The rationale for the use of the different treatments was to determine if ontogeny of adrenoceptors was under neuronal and/or hormonal control. Chemical sympathectomy by 6-OHDA was performed to determine if this treatment could possibly delay maturation of the α_1-adrenoceptor mediated functions. Dexamethasone and triiodothyronine were injected to determine if these treatments could possibly enhance maturation..

2.3 Methods

2.3.1 Functional studies:

Sprague-Dawley male rats aged 0.5, 1, 2, 3, 6 and 10 weeks were decapitated. Chest was opened and hearts were quickly removed and placed in oxygenated Krebs buffer of the following composition (mM): $NaHCO_3$ 25, NaCl 117, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.18, KH_2PO_4 1.18, dextrose 10, and ethylenediamine tetraacetic acid 0.03, as previously described (Varma and Yue, 1986). Whole ventricles from 0.5 and 1 week old pups and right ventricular strips from older rats were set up in 50 ml tissue baths at 32°C containing Krebs buffer aerated with 95% O_2 and 5% CO_2. The reason for using 1.8 mM instead of the usual 2.5 mM calcium and 32°C instead of the usual 37°C temperature was to prevent a very high basal contraction, which reduces the magnitude of maximal responses to inotropic agents.

In most studies, preparations were electrically stimulated at 1 Hz, 5 ms pulse duration and 1.5 times the threshold voltage by bipolar platinum electrodes. Tension was adjusted in each preparation to yield maximal basal contraction. Preparations were allowed to equilibrate for 60-90 min during which time they were washed with fresh Krebs buffer every 15-20 min. Since a majority of neonatal hearts exhibited automaticity, lidocaine (10 μM) was added to all preparations before the addition of drugs. Isometric contractions were recorded by means of a Grass force-displacement

transducers (FTO3C) on a Grass polygraph (Model 7).

Dose-response curves were determined cumulatively by increasing drug concentration by a factor of approximately 3; the next higher dose was added after the effect of the preceding concentration had reached a plateau (Varma and Yue, 1986). One preparation was used for only one dose-response curve. Some studies were done with the 1 week old and adult rat ventricle in the presence of 1 μM prazosin or 1 μM propranolol, which was added to the bath 20 mins prior to the addition of methoxamine. A similar study was done with isoprenaline. In all experiments, the maximal inotropic response to calcium was determined after the completion of dose-response curves.

In some studies ventricular preparations from 1- and 10-week rats were stimulated at different frequencies (0.5, 1 and 2 Hz) and inotropic responses to methoxamine were determined. This was done to determine if differences in the inotropic effects of methoxamine in young and adult hearts were due to differences in frequency-tension relationship.

In other studies, pups were injected i.p. with dexamethasone (1 mg/kg), 6-hydroxydopamine (150 mg/kg) or triiodothyronine (0.2 mg/kg) starting from the day of birth. Control pups were injected with the same volume of saline. On the seventh day pups were sacrificed and ventricles utilized for the determination of dose-response curves.

Quantitation of the response:

Basal contractions of different preparations were variable. Therefore inotropic effects of drugs were not calculated as absolute values. Instead, inotropic responses to sympathomimetic amines were calculated as percent increase over basal contraction (contraction before starting determination of dose-response curves) as follows:

% Increase = [(maximal contraction after a given drug concentration - basal contraction) / (basal contraction)] x 100.

Potency (EC_{50}) was calculated as -log Molar concentration of the test agent causing 50% of the maximal response where the maximal response of each preparation was treated as 100%. EC_{50} was derived from the regression line of the probit of the percent response versus -log molar concentration of the drug as previously described (Potvin and Varma, 1990).

Inotropic efficacy was the maximal increase over basal contractions. In addition to these calculations, maximal response to methoxamine and isoprenaline were also calculated relative to the maximal effect of calcium, usually 4 mM. Since in many experiments the maximal response to methoxamine as a percent of maximal response to calcium was less than 50%, EC_{25} (concentration causing 25% of maximal response to calcium) instead of EC_{50} was calculated.

2.3.2 Radioligand binding studies:

Membrane preparation

Rats aged 1 week and 10 weeks were decapitated. Hearts were quickly removed and dissected free of pericardium, atria, fat and large vessels. Crude membrane preparations were made according to Baker et al (1980) as previously used in our laboratory (Varma & Yue, 1986; Benfey et al., 1983). Ventricles were blotted and weighed, washed in saline and placed in 15 ml 10 mM Tris-HCl buffer (pH 7.8). Tissues were then minced and homogenized by a Polytron homogenizer (speed set at number 10, for 1 x 10 sec bursts). The homogenate was diluted with an equal volume of 1 M KCl, left on ice for 15 minutes and centrifuged at 48,000 x g at 4°C for 15

min in a Beckman L8-M ultracentrifuge. The supernatant was discarded and the pellet was resuspended in 30 ml buffer containing 50 mM Tris-HCl, $MgCl_2$, and EDTA (pH 8), passed through 4 layers of cheese cloth and recentrifuged at 48,000 x g at 4°C for 15 min and this pellet was suspended in sufficient buffer to yield membrane preparations of 500 µg/ml protein.

α_1-Adrenoceptor binding assays:

Assays were done in duplicate using [^{3}H]-prazosin to label α_1-adrenoceptor binding sites. Phentolamine was used as displacer. Aliquots of membrane preparations (20 µg protein) were incubated in a total volume of 200µl with [^{3}H]-prazosin (2-20 nM) in the presence or absence 10 µM phentolamine for 20 minutes at room temperature.

ß-Adrenoceptor binding assays: Assays were done in duplicate using [^{3}H]-dihydroalprenolol as ligand and propranolol as displacer. Aliquots of 20 µg of membrane preparations were incubated in a total volume of 200 µl with [^{3}H]-dihydroalprenolol (2-20 nM) in the presence or absence of 10 µM propranolol for 20 minutes at room temperature.

Incubation was terminated by rapid vacuum filtration through Whatman glass fiber filters (GF/C) and 5-6 washes with ice cold incubation buffer. Filters were presoaked with the incubation buffer to reduce blanks. After the filtration, filters were dried under warm air and then placed in counting vials and 0.5 ml of water was added; vials were vigorously shaken for 30 min in a shaker-bath after which 4.5 ml of scintillation fluid (Beckman Readysafe) was then added for counting the radioactivity (LKB Rackbeta Model 1290).

The specific binding was calculated by subtracting non specific binding (binding in the presence of excess of the competitor) from the total binding (binding in the absence of competitors). Proteins were determined by the dye-binding technique (Bradford, 1976) using bovine serum albumin as the standard.

Analyses of the binding data for the maximum number of binding sites (B_{max}) and the apparent dissociation constant (kD) were done by Scatchard plot using the non-linear regression LUNDON program.

2.3.3 Measurement of Inositol triphosphate (IP_3) accumulation:

Accumulation of IP_3 induced by the α_1-adrenoceptor agonist methoxamine was studied in 1 week old and adult rat ventricles. Ventricular pieces (wet weight 2-5 mg) were preincubated for 10 minutes at 32°C in Krebs buffer with 1.8 mM $CaCl_2$ and 10 mM LiCl and aerated with 95% O_2 and 5% CO_2. Tissues were then incubated with increasing concentrations of methoxamine for 7.5 minutes. It was previously determined in time-course studies that maximal IP_3 accumulation occurred between 7-8 min. Reaction was terminated with the addition of 100 µL of 20% perchloric acid and tissues were left on ice for 20 minutes. Tissues were homogenized with two 20 second bursts and centrifuged at 2,000 x g for 10 min. The pH of the supernatant was adjusted to 7.5 using a solution containing 1 5 mM KOH and 60 mM HEPES. The supernatant was then recentrifuged and the resultant supernatant was freeze-dried. Samples were reconstituted using a buffer containing 1M Tris-HCL, 4mM EDTA and 4 mg/ml BSA (pH 9). The IP_3 levei was measured for each sample using a competitive binding assay kit (Amersham, Mississauga, Ont.), which uses a bovine derived adrenal binding protein specific for IP_3. Protein content of samples was determined by the dye-binding technique (Bradford, 1976) using bovine serum albumin as the standard.

2.4 Statistics

Multiple means were analyzed by one-way analysis of variance followed by comparison of each pair in the group (Bonferroni) and the two means were compared by student's t-test for unpaired data. A probability of less than 0.05 was assumed to denote significant differences. Throughout this paper, means ± S.E. are presented.

SECTION 3: RESULTS

3.1 Inotropic effects of sympathomimetic agents

The majority of ventricular preparations from neonatal rats exhibited automaticity. Automaticity was rare in ventricular preparations from 3 weeks and older rats. In order to perform studies under uniform conditions, lidocaine (10 μM) was added to all preparations approximately 10 minutes prior to the start of the experiments. Lidocaine did not alter the basal contractions nor did it alter the inotropic effects of other agents, which was tested in preparations not exhibiting automaticity.

3.1.1 Inotropic effects of Methoxamine:

Methoxamine, a selective α_1-adrenoceptor agonist, produced a concentration-dependent inotropic effect on the rat ventricular preparations (Fig. 1). The inotropic effect was induced at bath concentrations ranging from 1 μM to 0.1 mM. The maximal inotropic effect produced by methoxamine on ventricular preparations from rats aged 0.5-, 1- and 2-weeks was 119±11, 169±16 and 109±21 respectively (Table 1). In preparations from rats aged 3-, 6- and 10-weeks, the maximal inotropic effects of methoxamine were 71±11, 49±7 and 35±3 respectively. The maximal inotropic effect of methoxamine on preparations from rats aged 0.5-, 1- and 2-weeks was significantly greater ($p<0.05$) than on preparations from 3-, 6- and 10-weeks old animals. The inotropic potency of methoxamine in preparations from 0.5 and 1 week old pups was greater than in tissues from older rats (Table 1). Thus, the inotropic effect of methoxamine progressively decreased with age and reached adult levels at 3 weeks (Table 1).

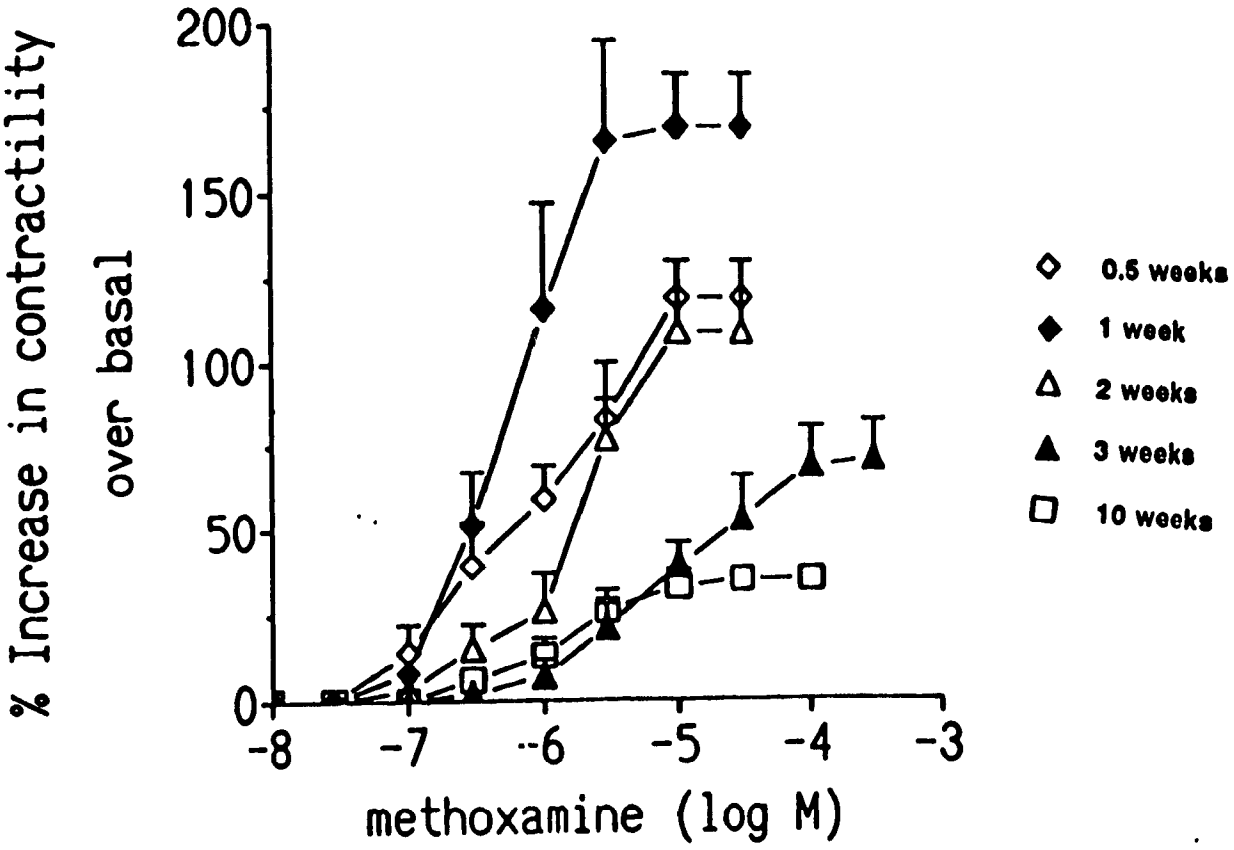

Figure 1. Inotropic concentration-response curves to methoxamine on electrically driven (1 Hz) ventricular preparations from rats of different age groups. The response is expressed as percent increase over basal contraction. Vertical bars represent standard error of the means (n=7-12).

Table 1. Inotropic efficacy and potency of methoxamine on electrically driven (1 Hz) ventricular preparations from different age groups of rats

Age (weeks)	n	Maximal effect (%)	EC_{50} (-log M)
0.5	8	118±8*	6.21±0.20**
1	11	169±16*	5.88±0.05**
2	12	109±21*	5.30±0.25
3	7	71±11	5.22±0.15
6	5	49±7	5.25±0.05
10	9	35±3	5.54±0.19

Data are mean ± S.E.M

*Different ($p<0.05$) from the bottom three values.

** Different ($p<0.05$) from the bottom four values.

Table 2. Inotropic efficacy and potency of methoxamine on electrically driven (1 Hz) ventricular preparations from different age groups of rats

Age (weeks)	n	Maximal Effect (% of Ca^{2+})	EC_{25} (-log M)
0.5	8	72±5*	5.69±0.10*
1	11	59±4*	5.87±0.12*
2	12	48±12	5.36±0.39*
3	7	48±4	4.99±0.17
6	5	27±4	4.78±0.16
10	9	28±5	4.97±0.28

Maximal response to calcium was taken as 100%.

Different ($p < 0.05$) from all the bottom values without the asterisks.

The maximal inotropic effect of methoxamine relative to that to calcium was also greater in preparations from rats aged 0.5 to 3-weeks; the potency of methoxamine based on maximal response to calcium treated as 100% was greater on preparations from rats aged 0.5, 1, and 2 weeks than on preparations from 6 and 10 weeks old animals (Table 2).

3.1.2 Inotropic effects of Isoprenaline:

The inotropic effects of ß-adrenoceptor agonist isoprenaline were observed at concentrations ranging from 1 nM to 0.1 µM (Fig. 2). Isoprenaline produced concentration-dependent increases in ventricular contractions. Inotropic efficacy of isoprenaline on ventricular preparations from rats of different age groups did not differ significantly (Table 3). No significant difference was found in the inotropic potency of isoprenaline in the different age groups. The maximal inotropic effects of methoxamine and isoprenaline were comparable in ventricular preparations from rats aged 0.5-, 1- and 2-weeks. However, the maximal inotropic effect of isoprenaline was greater than that of methoxamine in tissues from rats 3 weeks of age or older. There was no significant age-dependent difference in the potency of isoprenaline (Tables 3 and 4).

3.1.3. A comparison of inotropic efficacy of methoxamine and isoprenaline

The maximal inotropic effect of methoxamine both as increase over basal (Table 5) and as percent of maximal to calcium (Table 6) were comparable to that of isoprenaline in ventricular preparations from rats up to 2 weeks of age. In preparations from older rats, the inotropic efficacy of methoxamine, as expected, was significantly less than that of isoprenaline (Tables 5 and 6).

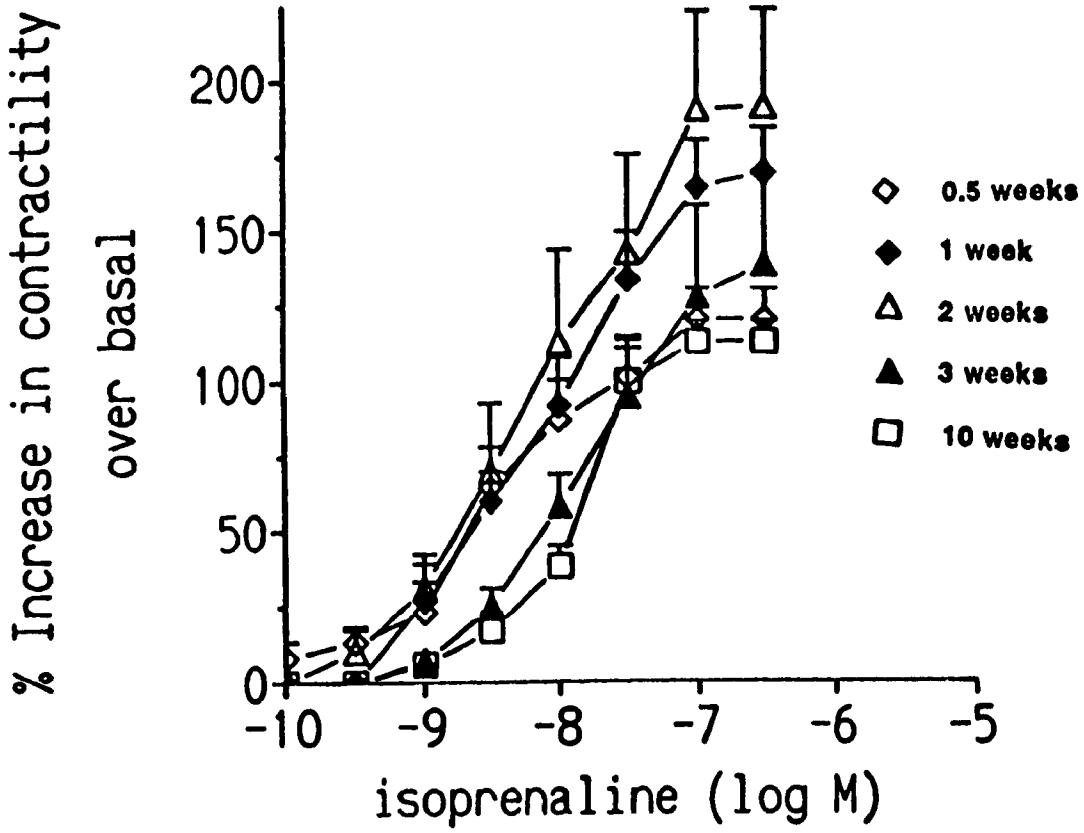

Figure 2. Inotropic concentration-response curves to isoprenaline on electrically driven (1 Hz) ventricular preparations from rats of different age groups. The response is expressed as percent increase over basal contraction. Vertical bars represent standard error of the means (n=5-11).

Table 3. Inotropic efficacy and potency of isoprenaline on electrically driven (1 Hz) ventricular preparations from different age groups of rats

Age weeks)	n	Maximal effect(%)	EC_{50} (-log M)
0.5	8	120±10	8.70±0.16
1	11	170±15	8.00±0.10
2	10	192±33	7.79±0.07
3	6	139±31	8.00±0.18
6	5	137±12	8.01±0.08
10	11	116±9	7.88±0.10

Data are mean ± S.E.M

Table 4. Inotropic efficacy and potency of isoprenaline on electrically driven (1 Hz) ventricular preparations from different age groups of rats.

Age (weeks)	n	Maximal effect (% of Ca^{2+})	EC_{25} (-log M)
0.5	8	83±5	8.85±0.17
1	11	64±6	8.22±0.11
2	10	71±10	8.13±0.18
3	6	89±2	8.86±0.09
6	5	93±4	8.77±0.12
10	11	98±2	8.35±0.09

Maximal response to calcium was taken as 100%.

Table 5. A comparison of the maximal inotropic activity of methoxamine and isoprenaline on electrically driven (1 Hz) ventricular strips from different age groups of rats

Age	Maximal increase in contractility over basal (%)			
(weeks)	n	Methoxamine	n	Isoprenaline
0.5	8	118±8	8	120±10
1	11	169±16	11	170±15
2	12	109±21	10	192±33*
3	7	71±11	6	139±31*
6	5	49±7	5	137±12*
10	9	35±3	11	116±9*

* Different ($p < 0.05$) from the corresponding value for methoxamine.

Table 6. A comparison of the inotropic efficacy of methoxamine and isoprenaline relative to that to calcium on electrically driven (1 Hz) ventricular strips from different age groups of rats

Age (weeks)	n	Maximal response (% of maximal to calcium) Methoxamine	Isoprenaline
0.5	8	72±5	83±5
1	11	59±4	64±6
2	10-12	48±12	71±10
3	6-7	48±4	89±2*
6	5	27±4	93±4*
10	9	28±5	98±2*

Different ($p < 0.05$) from the corresponding value for methoxamine.

3.1.4 Inotropic effects of phenylephrine:

The inotropic effects of another α_1-adrenoceptor agonist, phenylephrine, were studied on 1- and 10-week old rat ventricular preparations. Concentration-dependent increases in its inotropic effect were observed from 10 nM to 0.1 μM on 1-week old rat ventricular preparations. In the 10-week old rat ventricular preparations, concentrations causing inotropic effects ranged from 100 nM to 1 μM (Fig 3) The maximal inotropic effects in 1- and 10-week old rat ventricular preparations were 113±12% and 39±7% respectively, and significantly different from each other (Table 7). Also, the inotropic potency (EC_{50}, -log molar) was significantly ($p<0.05$) greater in the 1-week old rat ventricular preparations than in ventricular preparations from 10-week old animals (7.45±0.30 and 6.11±0.10, respectively). The EC_{25} of phenylephrine based on maximal response to calcium in 1-week old rat ventricular preparations was significantly lower than in the 10-week old (Table 8) again indicating that phenylephrine was more potent in neonatal than in adult rat ventricles.

3.1.5 Inotropic effects of noradrenaline:

Noradrenaline exerts its inotropic effect via β_1-adrenoceptors. In the present experiments noradrenaline behaved like isoprenaline with respect to inotropic effects in young and old rat hearts. Noradrenaline produced concentration-dependent inotropic effect both in 1- and 10-week old rat ventricular preparations (Fig. 4). The maximal inotropic effect of noradrenaline was 138±17 and 144±19, respectively, in the 1- and 10-week old rat ventricular preparations and the EC_{50} was 7.45±0.30 and 6.11±0.10 respectively (Table 7). Unlike isoprenaline, noradrenaline was more potent on preparations from 1 week old than on preparations from 10-week old rats (Table 7 and 8).

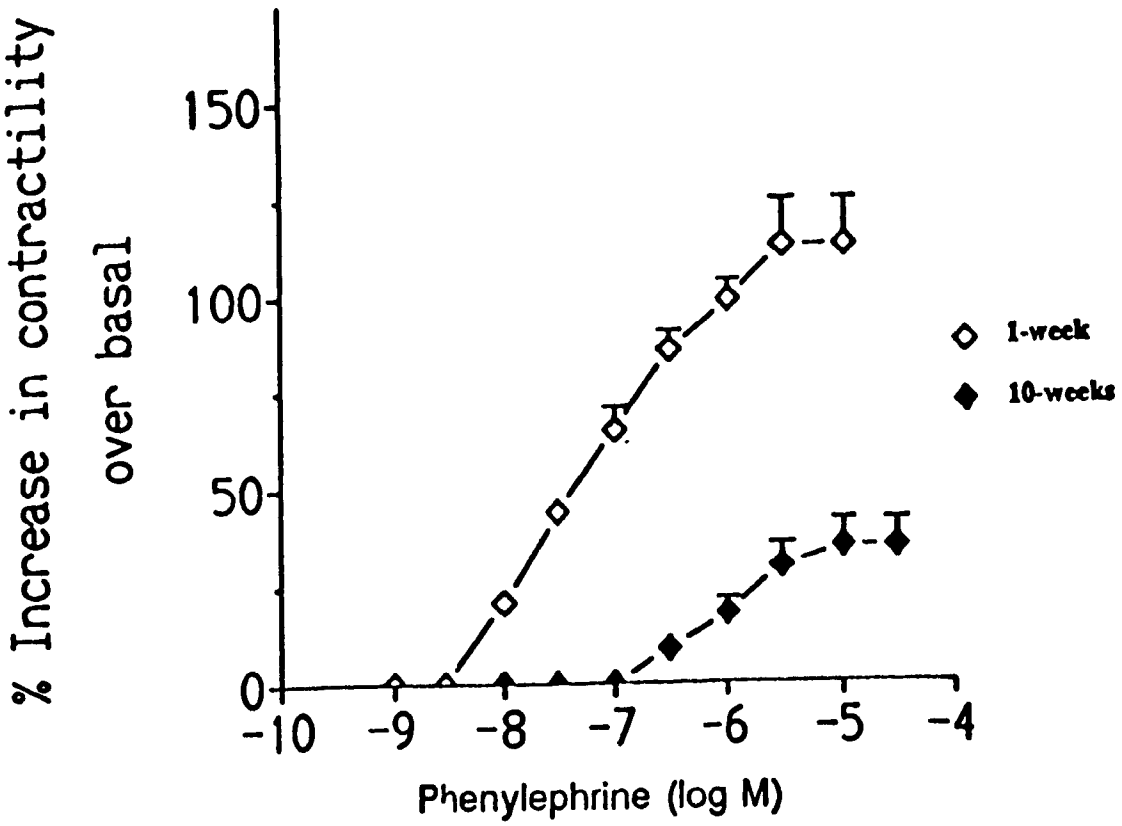

Figure 3. Inotropic concentration-response curves to phenylephrine on electrically driven (1 Hz) ventricular preparations from 1- and 10-week old rats. The response is expressed as percent increase over basal contraction. Vertical bars represent standard error of the means (n=4-5).

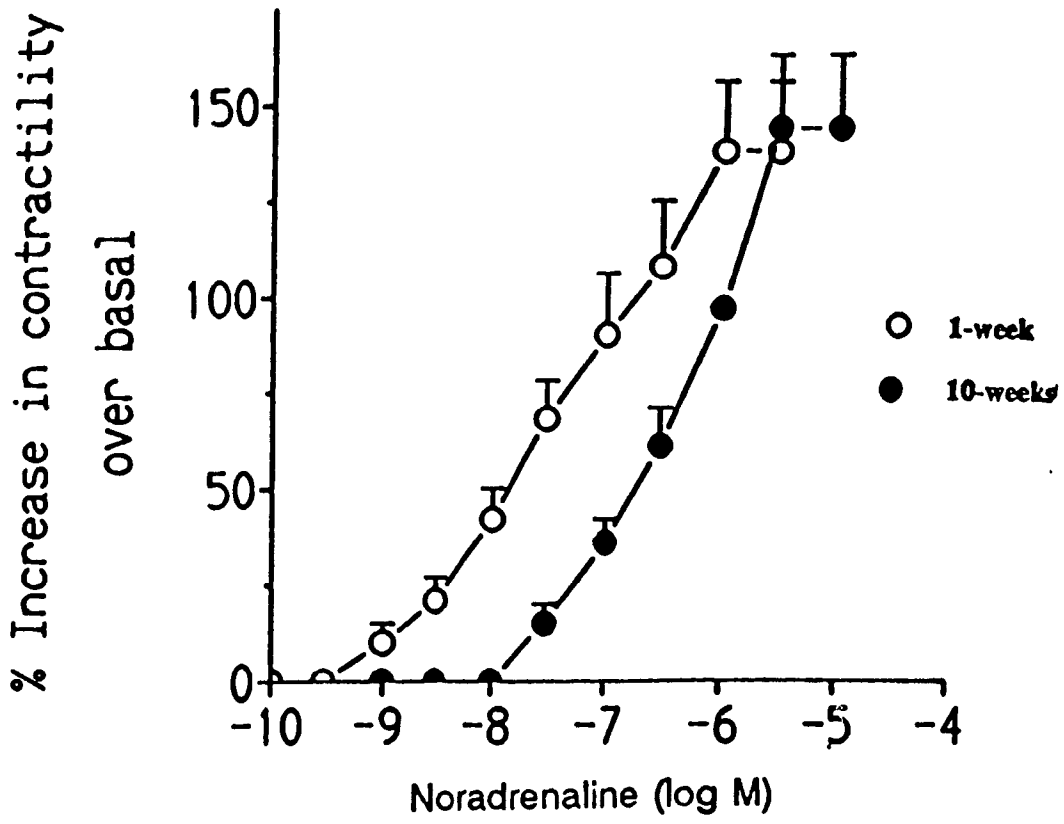

Figure 4. Inotropic concentration-response curves to noradrenaline on electrically driven (1 Hz) ventricular preparations from 1- and 10-week old rats. The response is expressed as percent increase over basal contraction. Vertical bars represent standard error of the means (n=4-5).

Table 7. Inotropic efficacy and potency of methoxamine, phenylephrine, noradrenaline and isoprenaline on electrically driven (1 Hz) ventricular preparations from 1- and 10-week old rats

Agent	Maximal effect (%)		EC_{50} (-log M)	
	1-Week	Adult	1-Week	Adult
Methoxamine	169±16	35±3*	5.88±0.05	5.54±0.19*
Phenylephrine	113±12	39±7*	7.45±0.30	6.11±0.10*
Noradrenaline	138±17	144±19	7.62±0.13	6.52±0.06*
Isoprenaline	120±11	116±9	8.00±0.10	7.88±0.10

Data are mean ± S.E.M

*Different ($p<0.05$) from corresponding values on the left; n= 4-11.

Table 8. Inotropic efficacy and potency of methoxamine, phenylephrine, noradrenaline and isoprenaline on electrically driven (1 Hz) ventricular preparations from 1- and 10-week old rats, based on maximal response to calcium

Agent	Maximal effect (% of Ca^{2+})		EC_{25} (-log M)	
	1-Week	Adult	1-Week	Adult
Methoxamine	72±5	28±5*	5.69±0.10	4.97±0.28
Phenylephrine	60±2	29±7*	7.34±0.21	5.25±0.31*
Noradrenaline	80±7	88±8	8.13±0.29	6.87±0.07*
Isoprenaline	83±5	98±2*	8.85±0.17	8.35±0.09

Data are mean ± S.E.M

*Different ($p<0.05$) from corresponding values on the left; n=4-11

3.2.1 Effect of frequency of stimulation on the inotropic activity of methoxamine:

Concentration-response curve to methoxamine on preparations from 1 week and 10 week old rats stimulated at 0.5 (Fig. 5), 1 (Fig. 6) and 2 (Fig. 7) Hz indicated that methoxamine was more effective on ventricles from 1 week old than 10-week old rats at all frequencies. The maximal inotropic effect of methoxamine in preparations driven at 0.5, 1 and 2 Hz from the 1-week old rat ventricle was (%) 124±16, 78±12 and 73±12 respectively where as that from the 10-week old was (%) 92±15, 35±3 and 35±7 respectively (Table 9).

Inotropic effects of methoxamine based on the maximal response to calcium in the 1-week old rat ventricular preparations driven at 0.5, 1 and 2 Hz were 65±9, 68±7 and 64±7 respectively (Table 9). The inotropic effects of methoxamine based on the maximal response to calcium were 38±6, 28±5 and 41±10 (Table 9). The inotropic responses of the 1-week old rat ventricular preparations driven at the three frequencies was significantly higher than those of the 10-week old rat preparations. It should be pointed out that many preparations from neonatal animals driven at 0.5 Hz were discarded because of automaticity despite the presence of lidocaine in the bath.

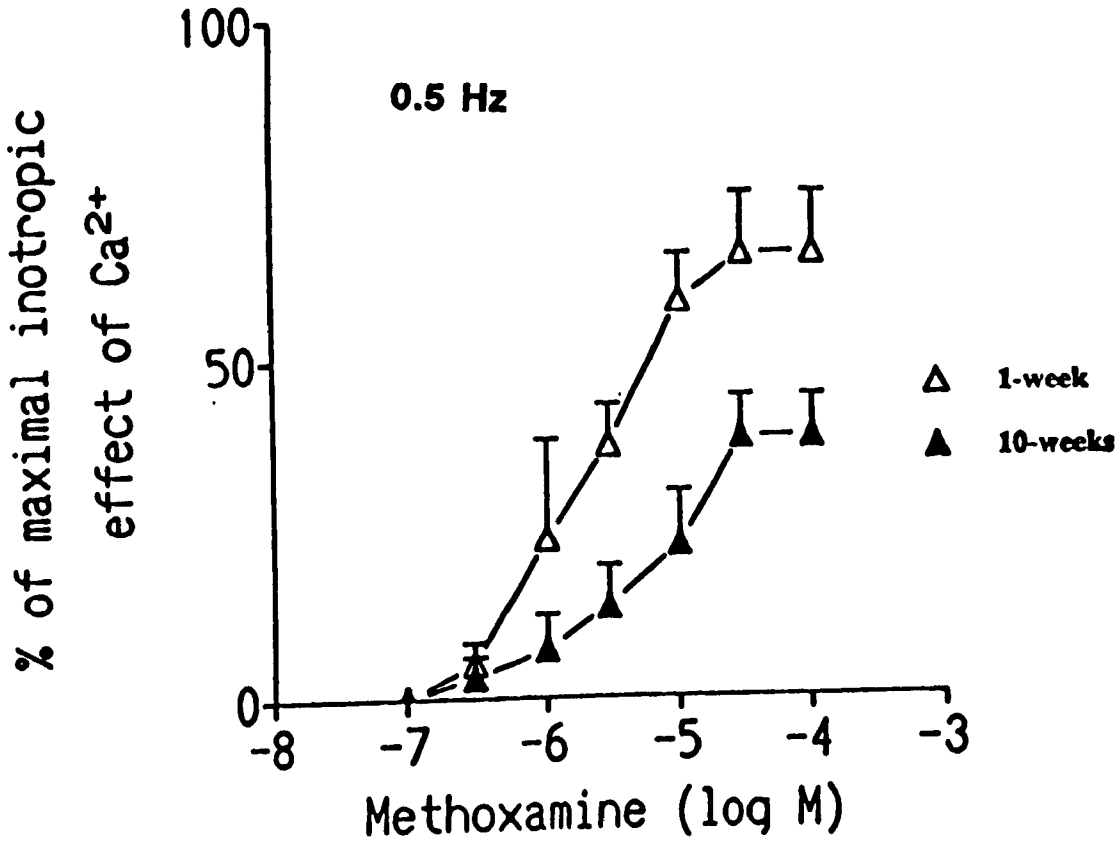

Figure 5. Inotropic concentration-response curves to methoxamine, based on the percent of maximal response to calcium from the 1- and 10-week old rat ventricular preparations **driven at 0.5 Hz.**

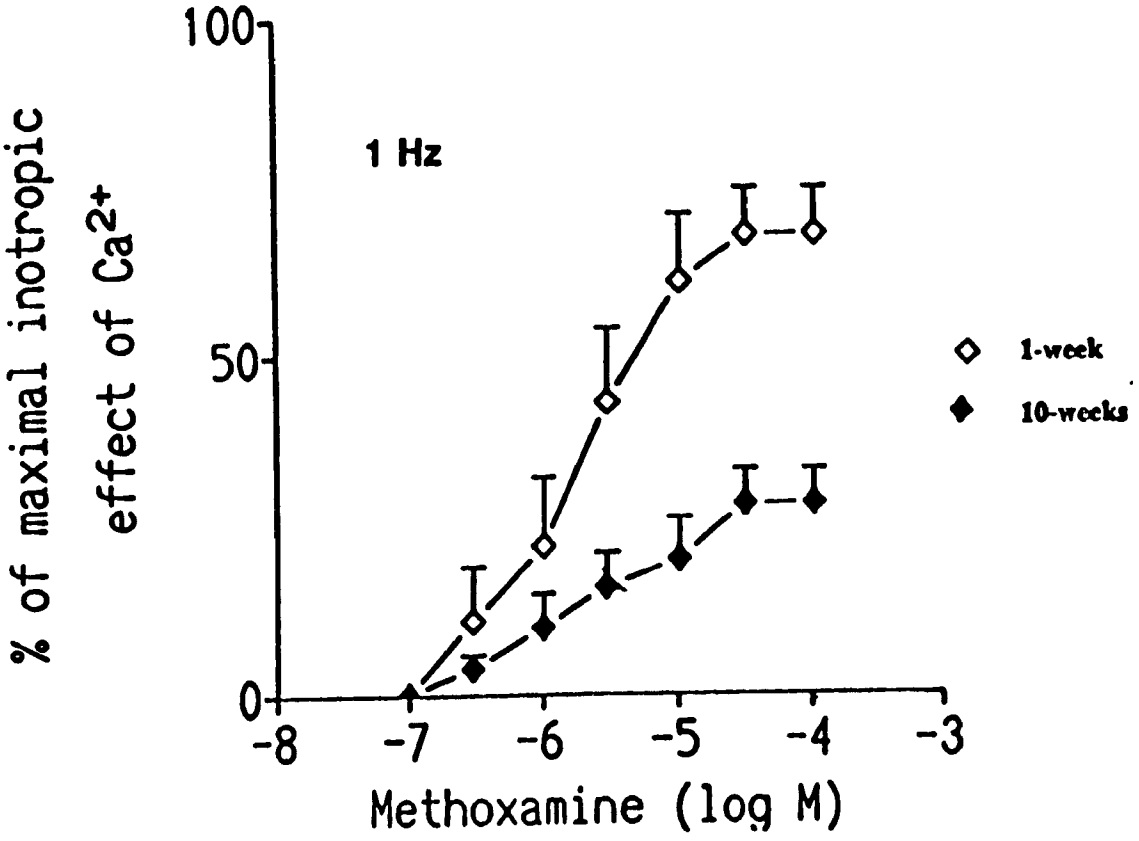

Figure 6. Inotropic concentration-response curves to methoxamine, based on the percent of maximal response to calcium in the 1- and 10-week old rat ventricular preparations **driven at 1 Hz.**

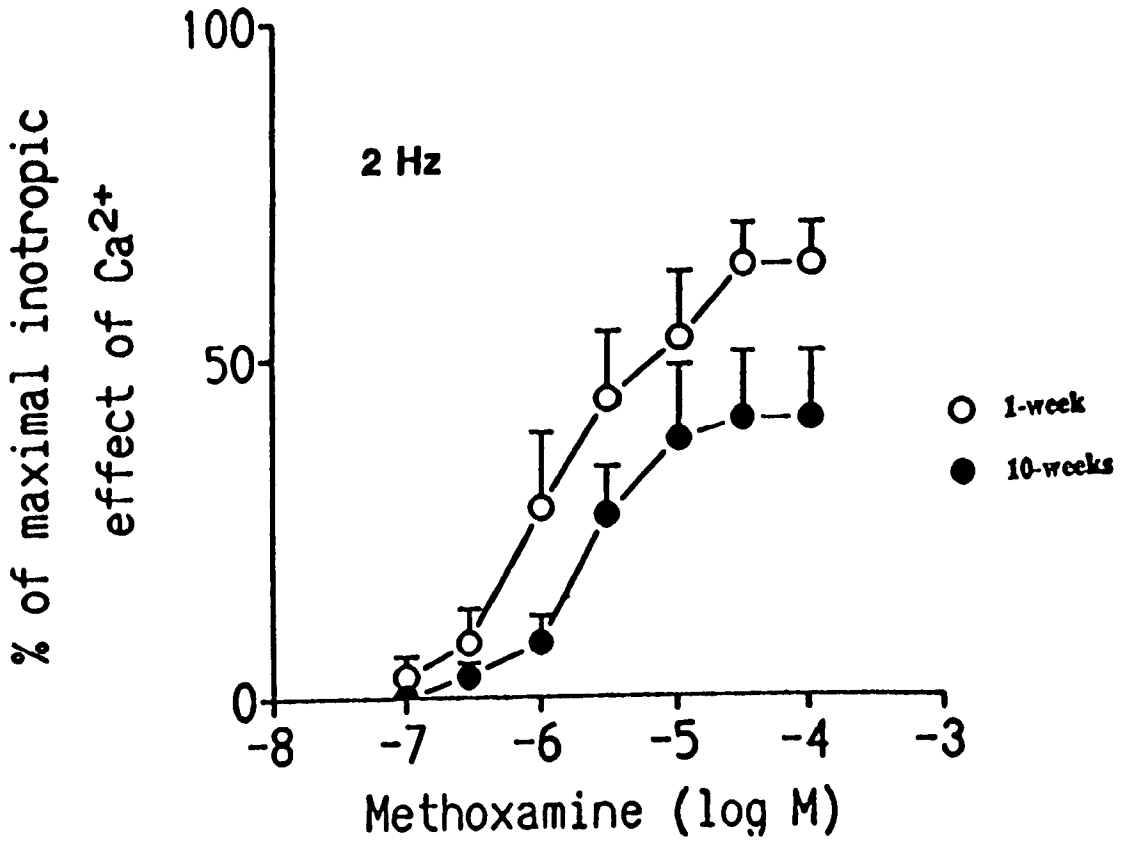

Figure 7. Inotropic concentration-response curves to methoxamine, based on the percent of maximal response to calcium in the 1- and 10-week old rat ventricular preparations **driven at 2 Hz.**

Table 9. Frequency-dependent inotropic effect of methoxamine from 1- and 10-week old rats.

Frequency (Hz)	Maximal effect (%)		% of Ca^{2+} response	
	1-Week	10-Week	1-Week	10-Week
0.5	124±16	92±15	65±9	38±6*
1	78±12	35±3*	68±7	28±5*
2	73±12	35±7*	64±7	41±10*

* Different ($p < 0.05$) from the corresponding value for 1-week. Data are means ± S.E.M. of 6-12 experiments.

3.3 Effects of adrenoceptor antagonists

3.3.1 Effect of adrenoceptor antagonists on the inotropic effect of methoxamine:

The effect of propranolol, a nonselective ß-adrenoceptor antagonist and prazosin, a selective α_1-adrenoceptor antagonist were studied on the inotropic effect of methoxamine on ventricular preparations from 1 week-old (Fig 8) and 10-week old (Fig. 9) rats. The bath concentrations of propranolol and prazosin were 0.1 µM. They were added to the bath 20 minutes prior to the addition of the agonist.

The effect of methoxamine was not blocked by propranolol on ventricular strips both from 1 week and 10-week old rats (Fig. 8). On the other hand, prazosin, the selective α_1-adrenoceptor antagonist, caused a parallel shift of the concentration-response curve to the right on preparations both from 1-week old (Fig. 8) and 10-weeks old (Fig. 9) rats.

3.3.2 Effect of adrenoceptor antagonists on the inotropic effects of isoprenaline:

Effect of prazosin (0.1 µM) and propranolol (0.1 µM) were studied on the inotropic effect of isoprenaline on ventricular preparations from rats aged 1 week and 10 weeks. The antagonists were added to the bath 20 minutes before starting the construction of concentration-response curves to isoprenaline.

Prazosin did not block the inotropic effect of isoprenaline (Fig. 10). In contrast, propranolol, the nonselective ß-adrenoceptor antagonist, caused a parallel shift to the right of the concentration response curves to isoprenaline on preparations from both 1-week old (Fig. 10) and 10-weeks old (Fig. 11) rats.

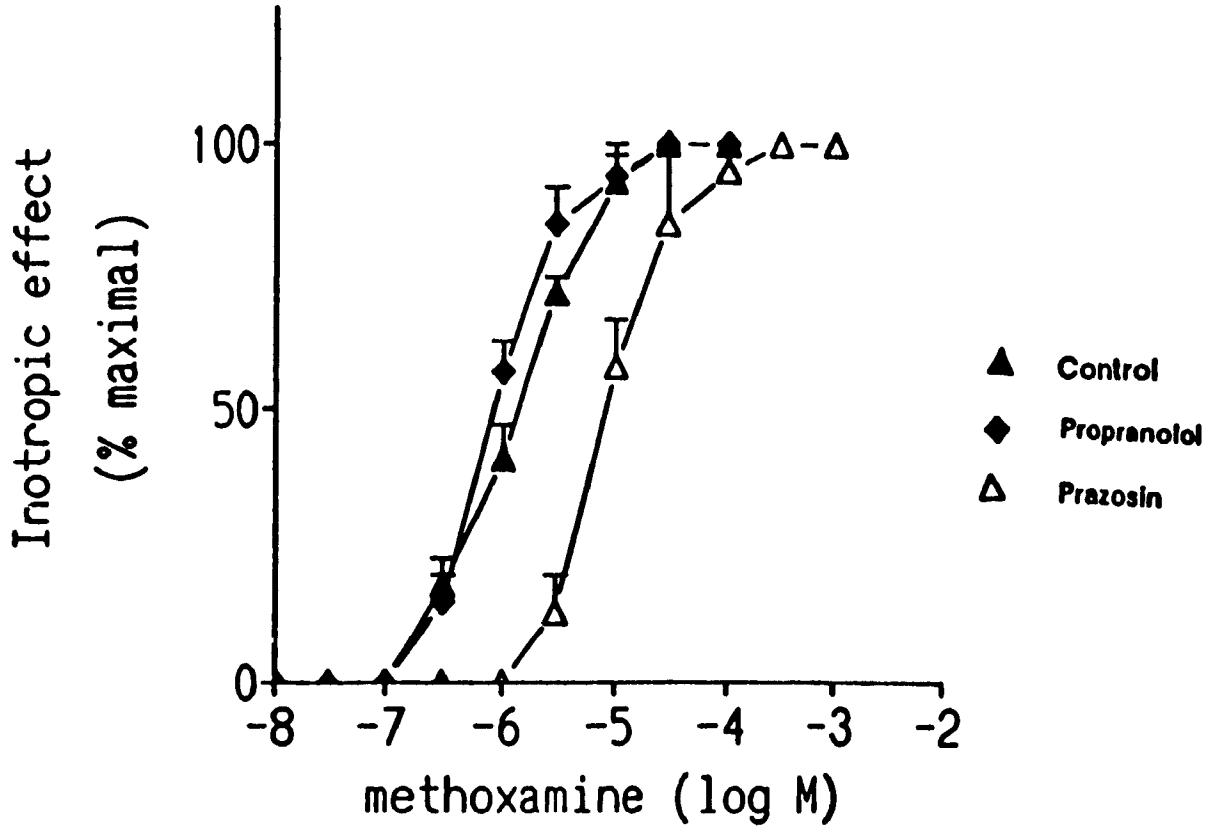

Figure 8. Effect of prazosin (0.1 μM, 20 min contact) and propranolol (0.1 μM, 20 min contact) on the inotropic effect of methoxamine on electrically driven (1 Hz) ventricular preparations from 1-week old rats. Vertical bars represent standard error of the means (n=4).

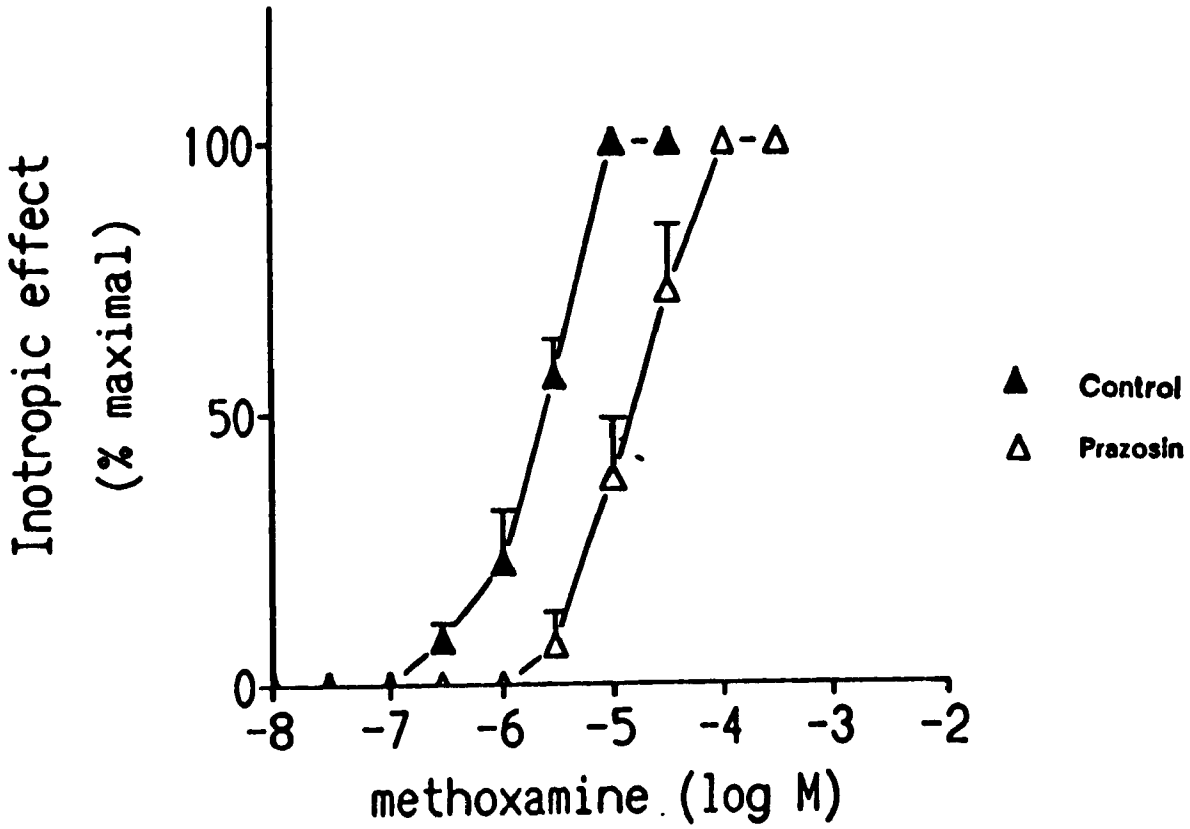

Figure 9. Antagonism by prazosin (0.1 μM, 20 min contact) of the methoxamine-induced positive inotropic effect on electrically driven (1 Hz) ventricular preparations from 10-week old rats. Vertical bars represent standard error of the means (n=4).

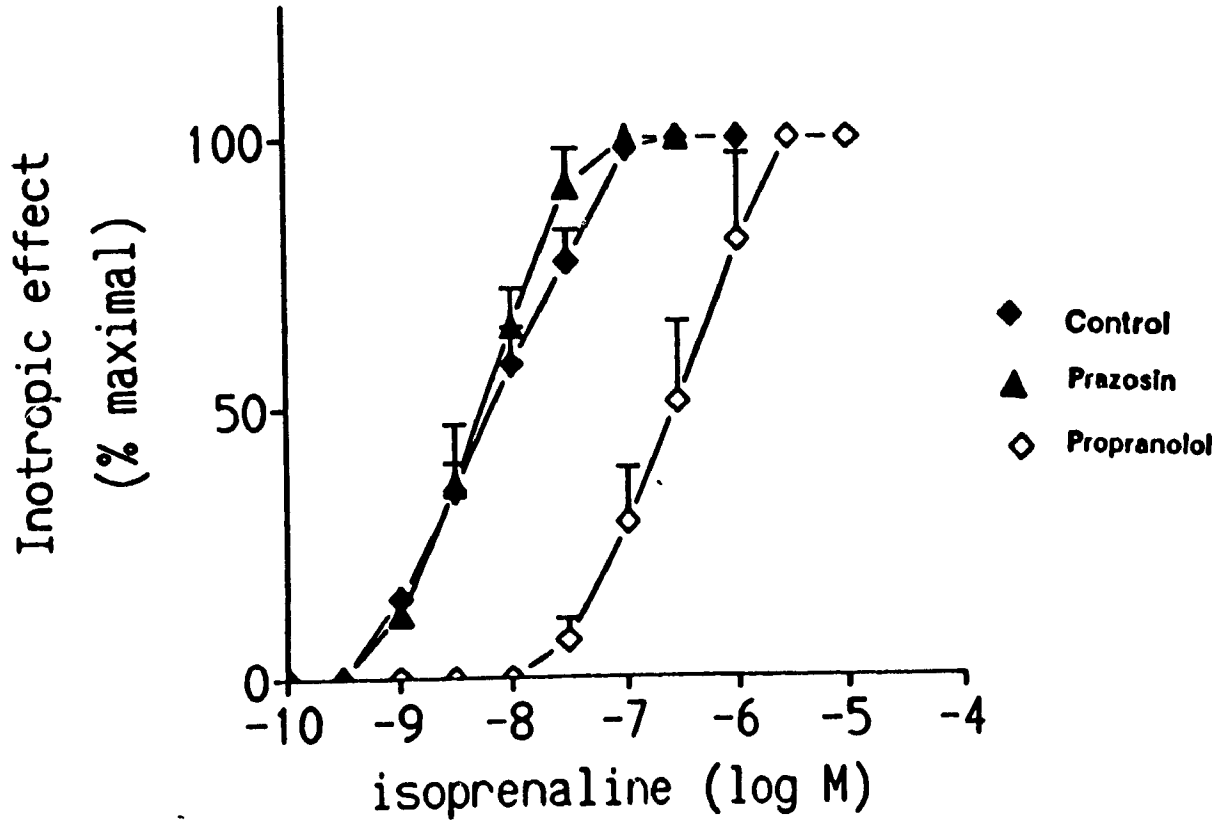

Figure 10. Effect of propranolol (0.1 μM, 20 min contact) and prazosin (0.1 μM, 20 min contact) on the inotropic effect of isoprenaline on electrically driven (1 Hz) ventricular preparations from 1-week old rats. Vertical bars represent standard error of the means (n=4).

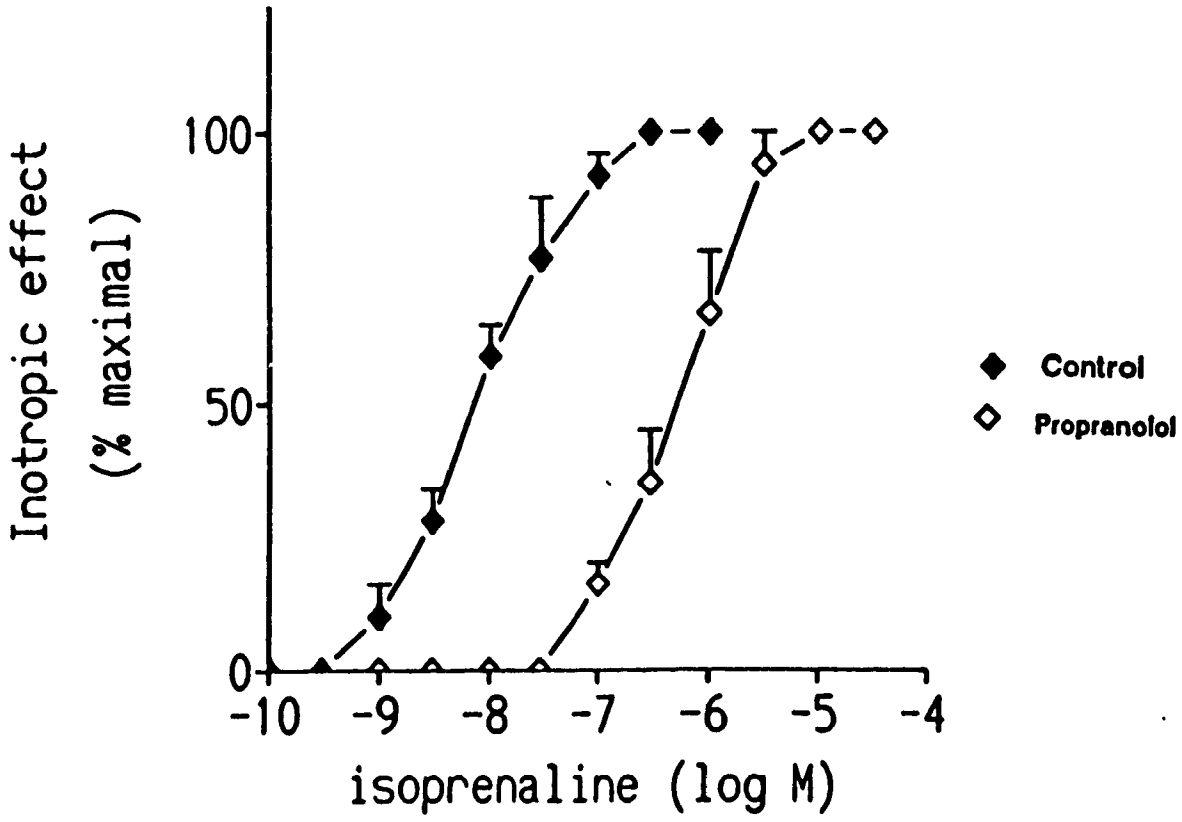

Figure 11. Antagonism by propranolol (0.1 μM, 20 min contact) of the isoprenaline-induced positive inotropic effect on electrically driven (1 Hz) ventricular preparations from 10-week old rats. Vertical bars represent standard of the means (n=4).

3.4 Influence of different treatments on the inotropic activity of methoxamine and isoprenaline

3.4.1 Influence of 6-hydroxydopamine (6-OHDA) treatment:

The effect of 6-OHDA treatment on inotropic activity of test agents was studied in 3 age groups. The maximal inotropic effect and potency of methoxamine were not significantly altered by injections of 6-OHDA (Table 10). On the other hand, the inotropic activity of isoprenaline was significantly ($p<0.05$) increased by 6-OHDA treatment (Table 11).

3.4.2 Influence of dexamethasone treatment:

Treatment with dexamethasone did not alter the maximal inotropic effect or the potency of methoxamine when compared to the control group (Table 12). Also dexamethasone administration to newborn rats did not alter the responses of the ventricular preparations to isoprenaline (Table 13).

3.4.3 Influence of triiodothyronine:

Treatment of newborn rats with triiodothyronine did not alter the responses of the ventricular strips to methoxamine (Table 12) and isoprenaline (Table 13).

Table 10. Influence of 6-hydroxydopamine (6-OHDA) treatment on the inotropic activity of methoxamine on electrically driven (1 Hz) ventricular preparations from different age group of rats

Treatment	Age (weeks)	Maximal effect (%)	EC_{50} (-log M)
None	1	169±16	5.88±0.05
6-OHDA[a]	1	188±16	5.51±0.10
None	3	71±12	5.22±0.15
6-OHDA[b]	3	71±5	5.50±0.25
None	6	35±3	5.54±0.19
6-OHDA[c]	6	59±15	5.55±0.07

Rat pups injected i.p. with 6-OHDA according to the following protocol: [a] days 1-3; [b] days 1-3 and again at 2 weeks; [c] days 1-3 and again at 2 and 4 weeks.

Data are mean ± S.E.M. (n = 4-6). No significant difference was found between the control and treated groups.

Table 11. Influence of 6-hydroxydopamine (6-OHDA) treatment on the inotropic activity of isoprenaline on electrically driven (1 Hz) ventricular preparations in different age groups of rats

Treatment	Age (weeks)	Maximal effect (%)	EC_{50} (-log M)
None	1	170±15	8.00±0.10
6-OHDA[a]	1	249±60*	8.62±0.13*
None	3	139±31	8.00±0.18
6-OHDA[b]	3	163±35*	9.22±0.12*
None	6	137±12	8.01±0.08
6-OHDA[c]	6	320±60*	8.57±0.16*

Rat pups injected i.p with 6-OHDA according to the following protocol: [a] days 1-3; [b] days 1-3 and again at 2 weeks of age; [c] days 1-3 and again at 2 and 4 weeks of age.

Data are means ± S.E M. (n= 4-6).

*Different ($p<0.05$) from the corresponding values for the untreated controls of the same age group.

Table 12. Influence of dexamethasone (Dexa) and triiodothyronine (T3) treatment on the inotropic activity of methoxamine on electrically driven (1 Hz) ventricular preparations from 1 week-old rat

Treatment	n	Maximal effect (%)	EC_{50} (-log M)
Saline	6	134±24	5.96±0.12
Dexa	8	150±26	6.05±0.09
T3	8	112±12	5.91±00.08

Data are mean ± S.E.M.

Neither treatments exerted significant effect.

Table 13. Influence of dexamethasone (Dexa) and triiodothyronine (T3) on the inotropic effect of isoprenaline on electrically driven (1 Hz) ventricular preparations from the 1-week old rat

Treatment	n	Maximal effect (%)	EC_{50} (-log M)
None	11	170±15	8.00±0.10
Dexa	5	136±23	7.96±0.11
T3	4	135±24	7.95±0.10

Data are mean ± S.E.M

Neither treatments exerted significant effects.

3.5 Adrenoceptor binding assays

Binding assays were done to compare the density and affinity of the adrenoceptors in the heart. Specific binding increased in proportion to the concentration until saturation was reached. B_{max} and K_d were calculated from scatchard plots using LUNDON program.

3.5.1 α_1-Adrenoceptor assay:

Labelling of α_1-adrenoceptors using [^{3}H]-prazosin as ligand and phentolamine as displacer revealed a single high affinity binding site. Maximal binding (B_{max}) expressed as fmol/mg protein in ventricular membrane preparations from 1- and 10-week old rats was 600±120 and 640±230, respectively, and not different from each other (Table 14). Similarly there was no significant difference in the apparent disassociation constant (K_d) expressed in nM in ventricular membranes from 1- and 10-week old rats (2.5±1.0 and 3.5±1.1 respectively). Figures 11 and 12 show saturation curves and Scatchard plots (inset) of representative experiments from [^{3}H]-prazosin binding on 1- and 10-week old rat ventricles.

3.5.2 ß-Adrenoceptor assay:

The binding of $ß_1$-adrenoceptor ligand, [^{3}H]-dihydroalprenolol, was with high affinity and to a single binding site. B_{max} for [^{3}H]-DHA binding to ventricular membranes, respectively, from 1- and 10-week old rats was (fmol/mg protein) 640±150 and 910±210 and K_d (nM) was 3.6±1.0 and 3.8±1.2. B_{max} and K_d did not change with age (Table 14 and 15). Figures 13 and 14 show saturation curves and Scatchard plots of representative experiments from [^{3}H]-DHA binding on 1- and 10-week old rat ventricles.

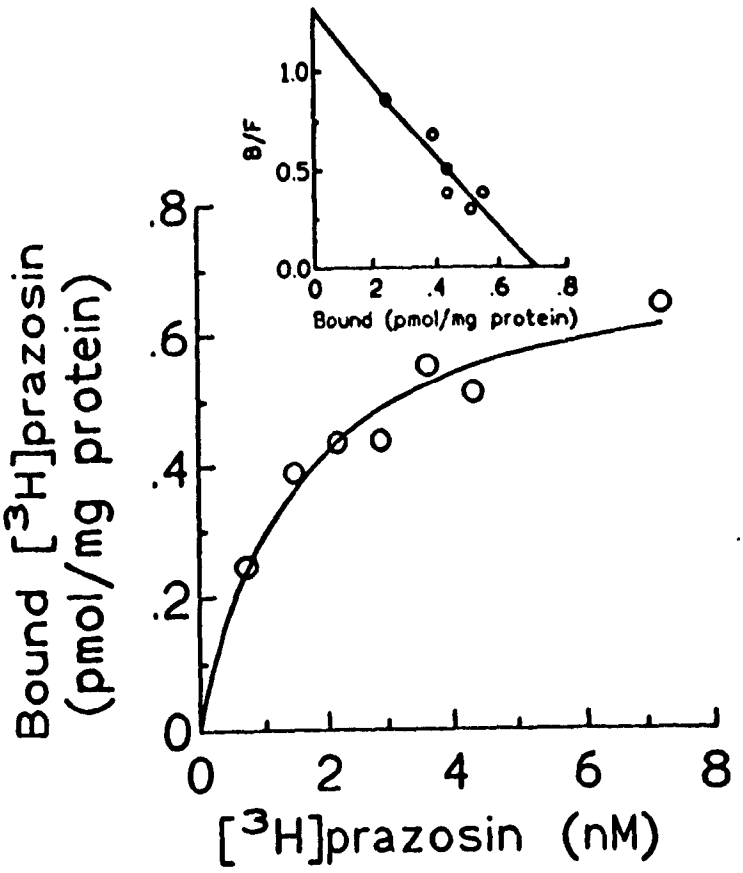

Figure 12. Representative curve for the specific binding of [^{3}H]-prazosin to ventricular membranes from the 1-week old rat, as a function of increasing concentrations of [^{3}H]-prazosin. Inset: Scatchard plot of specific [^{3}H]-prazosin binding to rat ventricular membranes.

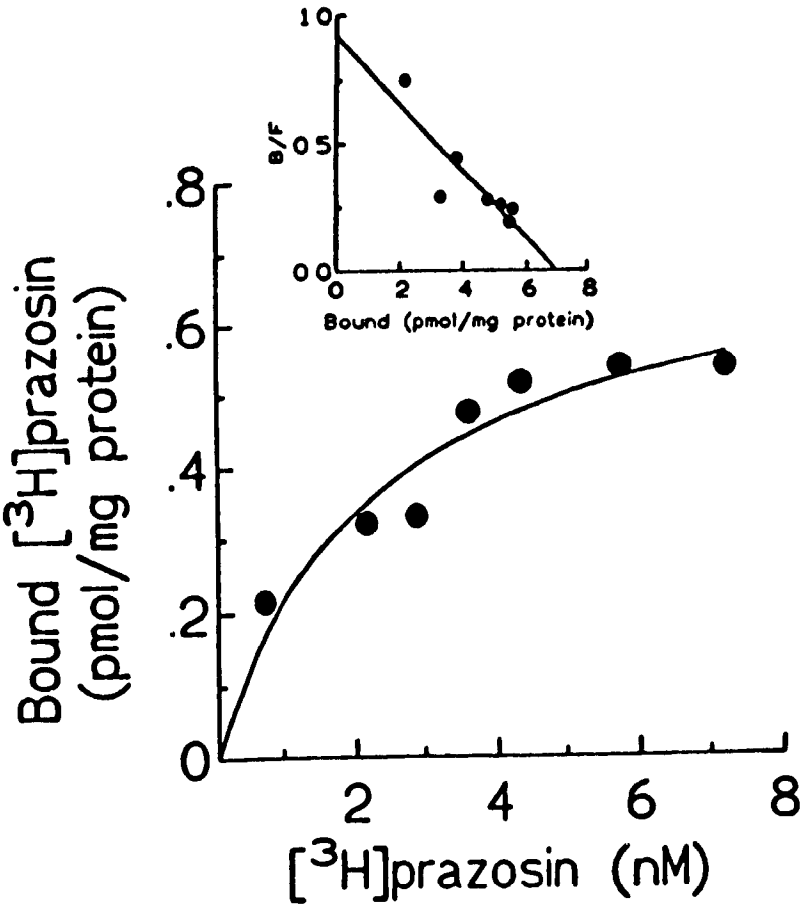

Figure 13. Representative curve for the specific binding of [^{3}H]-prazosin to ventricular membranes from the 10-week old rat, as a function of increasing concentrations of [^{3}H]-prazosin. Inset: Scatchard plot of specific [^{3}H]-prazosin binding to rat ventricular membranes.

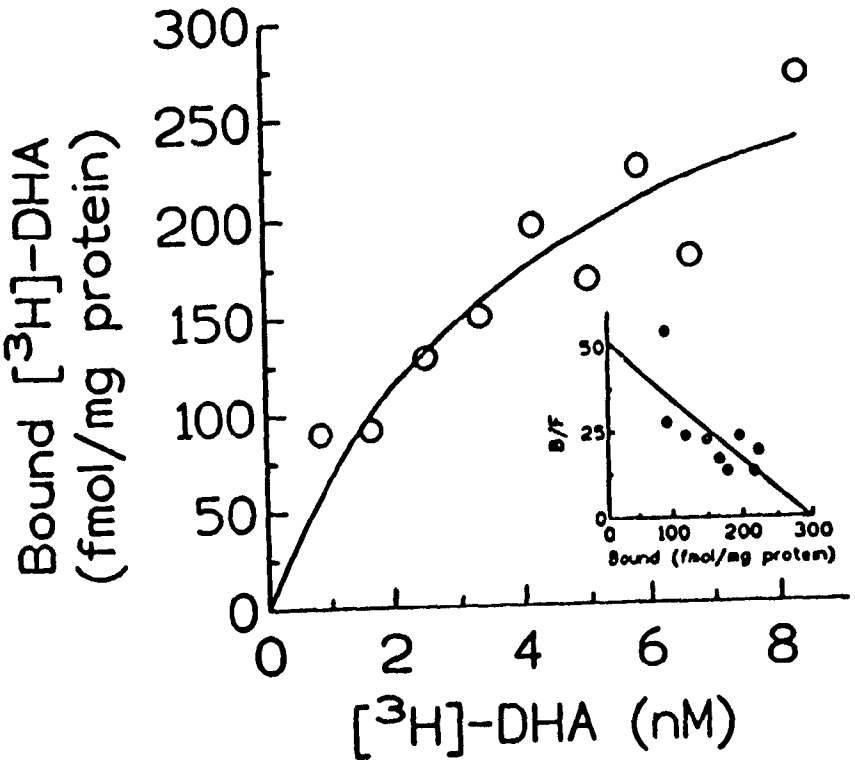

Figure 14. Representative curve for the specific binding of [^{3}H]-dihydroalprenolol to ventricular membranes from the 1-week old rat, as a function of increasing concentrations of [^{3}H]-dihydroalprenolol. Inset: Scatchard plot of specific [^{3}H]-dihydroalprenolol binding to rat ventricular membranes.

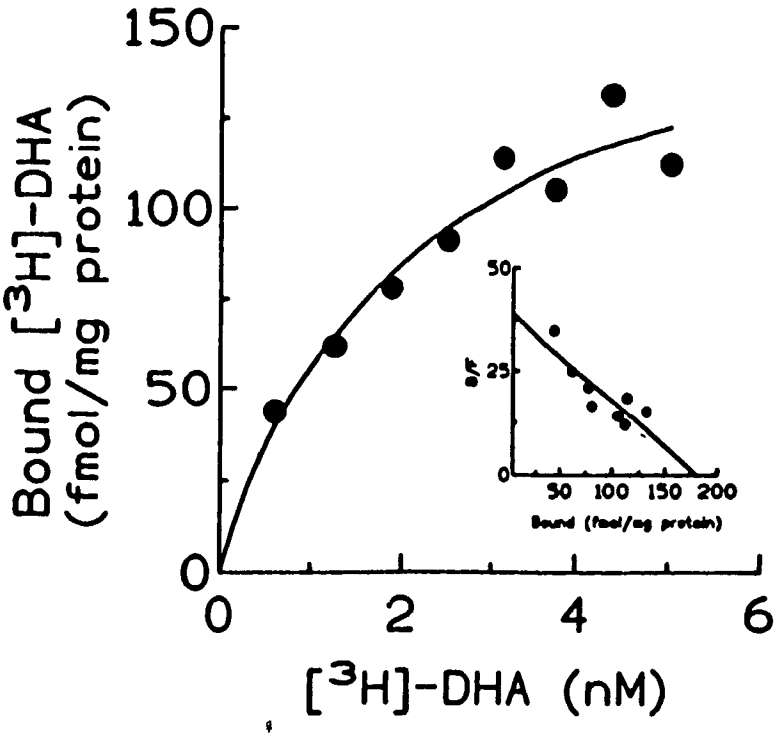

Figure 15. Representative curve for the specific binding of [^{3}H]-dihydroalprenolol to ventricular membranes from the 10-week old rat, as a function of increasing concentrations of [^{3}H]-dihydroalprenolol. Inset: Scatchard plot of specific [^{3}H]-dihydroalprenolol binding to rat ventricular membranes.

Table 14. Specific binding of [^{3}H]-prazosin to ventricular membranes from 1- and 10-week old rats

Age (weeks)	B_{max} (fmol/mg protein)	k_d (nM)
1	600±120	2.5±1.0
10	640±230	3.5±1.1

Data are mean ± S.E.M.

No significant difference was found between the 1-week and 10-week old rat ventricular membranes.

n=4, each in duplicate.

Table 15. Specific binding of [^{3}H]dihydroalprenolol to ventricular membranes from 1-week and 10-weeks old rats

Age (weeks)	B_{max} (fmol/mg protein)	k_d (nM)
1	640±150	3.6±1.0
10	910±210	3.8±1.2

Data are means ± S.E.M.; n =4, each in duplicate.

No age-related significant difference in specific binding was found.

3.6 Effects of methoxamine on inositol 1,4,5 triphosphate (IP_3):

Basal IP_3 expressed as pmol/mg protein was 219±29 in the 1-week old rat ventricle and 145±66 in the 10-week old rat ventricle. No significant difference was found in the basal IP_3 production in the above two groups.

Methoxamine induced concentration-dependent increases in the accumulation of IP3 in the ventricular tissue from the 10-week old rat (Fig 15.). On the other hand, methoxamine-induced increases in IP_3 accumulation were not observed in the ventricular tissues from the 1-week old rat (Fig 15.).

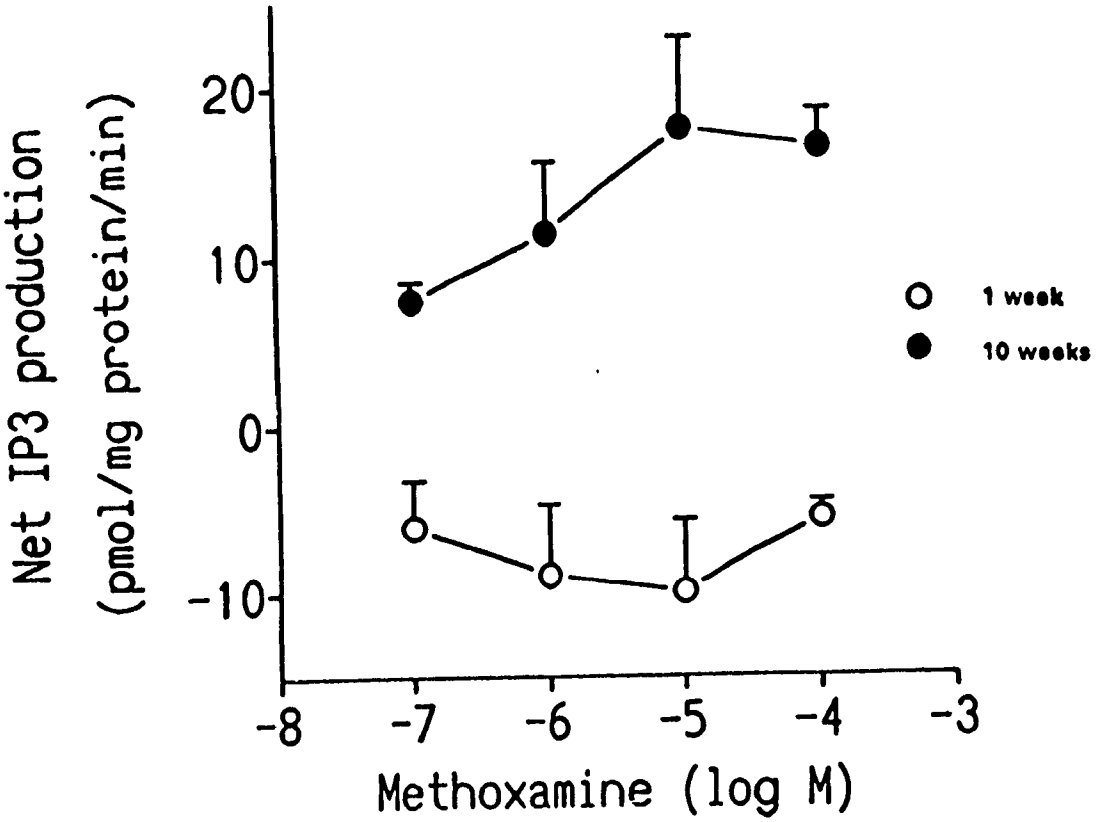

Figure 16. Effect of increasing concentrations of methoxamine on IP_3 accumulation in ventricles from the 1- and 10-week old rats. Vertical bars represent standard error of the means. Each value is derived from 4 separate experiments, each in duplicate.

SECTION 4: DISCUSSION

4.1 General discussion

The positive inotropic effect of catecholamines is primarily produced by an activation of β_1-adrenoceptors; α-adrenoceptors play a much smaller role. Consequently isoprenaline and endogenous catecholamines such as adrenaline, dopamine and noradrenaline increase myocardial contractility to a much greater extent than selective α-agonists such as methoxamine and phenylephrine. Noradrenaline, which is a potent α-adrenoceptor agonist produces its inotropic effect by activating β_1-adrenoceptors (Hoffman and Lefkowitz, 1990). However, the relative importance of myocardial α_1- and β_1-adrenoceptors in inotropic activity of sympathomimetic amines is subject to numerous factors such as the species (Kunos et al., 1978), temperature (Kunos and Nickerson, 1976) and thyroid state (Kunos et al., 1974, Ishac et al., 1983).

It was previously reported from our laboratory (Varma, 1991) that the ß-adrenoceptor agonist isoprenaline did not exert its full inotropic effect on neonatal rabbit ventricles. The α_1-adrenoceptor agonist, phenylephrine, too exerted little effect. However, the inotropic activity of isoprenaline in neonatal rabbit myocardial tissue in the presence of subeffective concentrations of phenylephrine was comparable to its activity in the adult rabbits On the basis of these observations, it was hypothesized that there might exist some functional link between α and ß-adrenoceptors in early life such that both need to be activated in order to elicit full effects of β_1-adrenoceptor agonists (Varma, 1991). The present study was undertaken using a more convenient rat model to extend these observations (Varma, 1991) made in rabbits.

However, the inotropic response of the neonatal rat myocardium was quite different from that of the rabbit. The principal difference was found with respect to α_1-adrenoceptor agonists. Methoxamine, a highly selective α_1-adrenoceptor agonist was comparable to the β_1-agonist, isoprenaline, in causing positive inotropic effect on myocardium of rats up to 2 weeks of age. This was an unusual observation since the maximal inotropic effect of α_1-adrenoceptor agonists is generally considerably smaller than that of β_1-adrenoceptor agonists (Hoffman and Lefkowitz, 1990). This thesis reports studies to characterize these age-dependent differences in the inotropic effects of α_1- and β_1-adrenoceptor agonists.

More commonly encountered ontogenic differences exemplify relative lack of maturation of a system in early life. Such lack of maturation is also known for cardiovascular system. For example PGE_2 and $PGF_{2\alpha}$, which are potent vasoconstrictors exert little effect on the cerebral blood vessels of newborn pigs (Chemtob et al., 1989). Isoproterenol exerts little inotropic effect on neonatal rabbit myocardium (Artman et al., 1988; Varma, 1991). On the other hand, a decrease in vasorelaxant effect with age in rabbit (Chemtob et al., 1991) as well as in chronotropic and inotropic effects of noradrenaline and isoprenaline in rat hearts has also been reported (Standen, 1978; Shigenobu et al., 1988; Mackenzie and Standen, 1980; Tanaka and Shigenobu, 1990). However, ontogeny of responses to selective α_1-adrenoceptor agonists have not been studied in any detail.

Although α_1-adrenoceptor agonists are known to cause inotropic effects (Govier et al., 1966), the relative efficacy of these agents is small compared to that of β_1-adrenoceptor agonists such as isoprenaline and noradrenaline (Varma, and Yue, 1986). In this sense the finding that the α_1-adrenoceptor agonist, methoxamine, was as effective as the ß-adrenoceptor agonist isoprenaline

in rats up to 2 weeks of age was surprising. A progressive decrease in the response to methoxamine with age denotes that the maturation of α_1-adrenoceptor mediated inotropic functions is characterized by a decrease rather than an increase.

Methoxamine was used as an α_1-agonist in most studies. However, the conclusion that activation of α_1-adrenoceptors produces inotropic effects comparable to that produced by activation of β_1-adrenoceptors was supported by the observation that phenylephrine behaved like methoxamine and noradrenaline was similar to isoprenaline. A significantly greater inotropic effect of methoxamine on neonatal rat myocardium than on ventricles of adult rats seems to be related to receptor functions and not a change in the tension-rate relationship in neonatal hearts. The inotropic efficacy of methoxamine was similar at stimulus frequency of 0.5, 1 and 2 Hz. Moreover, cardiac rate in rats from birth to adulthood does not differ significantly and is approximately 300 - 400 beats/min (Seidler & Slotkin, 1979).

Our results with isoprenaline are in conformity with those of other workers (Shigenobu et al., 1988; Standen, 1978) who also found that the potency of isoprenaline did not differ in the 1-week old and adult rat ventricles. Because basal contractions of neonatal and adult hearts were different, this study also analyzed data on inotropic response as a percent of the maximal inotropic effect of calcium. Data so analyzed also reveal that the response to isoprenaline does not change with age. A lack of age-dependent change in the inotropic effects of isoprenaline and noradrenaline is consistent with the observation that the density of β_1-adrenoceptors also did not differ at 1 week and 10 weeks of age. Other workers also found that major differences in β-adrenoceptor densities occur during fetal life and remain relatively unchanged at least after the first post-natal week in rats (Tanaka and Shigenobu, 1990; Cros et al., 1988).

The major finding of this study was the marked inotropic effect of methoxamine on ventricular strips from rats up to 2 weeks of age relative to that found in tissues from rats 3 weeks of age and older. Moreover, the inotropic efficacy of methoxamine was comparable to that of isoprenaline on tissues from 0.5, 1 and 2 weeks of age. This is surprising as numerous studies have demonstrated that the inotropic efficacy and potency of full ß-adrenoceptor agonists such as isoprenaline, adrenaline and noradrenaline are greater than those of selective α_1-adrenoceptor agonists (Endoh and Blinks, 1988, Govier 1967, Ishac et al., 1983).

An unexpectedly high α_1-adrenoceptor mediated inotropic effect as observed in this study on ventricles of rats up to 2 weeks of age could be due to several factors. First, methoxamine might not be acting through $ß_1$- and not α_1-adrenoceptors although no clear example of this phenomenon has been reported in the literature. However, the inotropic effect of methoxamine was blocked by the selective α_1-adrenoceptor antagonist prazosin but not by the nonselective ß-adrenoceptor antagonist propranolol. This implies that the effects of methoxamine on neonatal myocardium was exerted through an activation of α_1- and not $ß_1$-adrenoceptors. As expected the inotropic effect of isoprenaline was antagonized by propranolol but not by prazosin. Studies with another selective α_1-adrenoceptor agonist phenylephrine also suggested that α_1-adrenoceptor-mediated effects were greater on ventricles from young rats relative to that on tissues from adult animals

There is some evidence, however, that α-adrenoceptor agonists can sensitize tissues to the effects of ß-adrenoceptor agonists. For example, stimulation of α-adrenoceptors by phenylephrine markedly potentiated the effect of isoprenaline in stimulating pineal serotonin N-acetyltransferase (Klein et al., 1983). Potentiation of the inotropic effect of isoprenaline by phenylephrine on neonatal rabbit myocardial tissues has been reported from this laboratory (Varma,

1991). It is thus possible that methoxamine somehow increases the effects of noradrenaline being spontaneously released from the nerve endings in the cardiac tissues. Moreover, rat myocardium can also synthesize adrenaline (Kennedy and Ziegler, 1991). Methoxamine could also act by potentiating the effects of adrenaline, which is more potent than noradrenaline on the heart (Ahlquist, 1948). However, several factors suggest that this is highly unlikely.

First of all sympathetic innervation to rat myocardium is less complete by two weeks of age and during this period it is continuously increasing (Seidler and Slotkin, 1979; Lau and Slotkin, 1979; Pappano, 1977). The source of spontaneously released noradrenaline could only be sympathetic nerve endings. Therefore the inotropic effect of methoxamine would be expected to be greater in myocardium of adult than of neonatal rats if it acted by sensitizing the tissue to endogenously released catecholamines. Furthermore, if the effects of methoxamine were indirect in this sense, propranolol should have been able to antagonize its effects, which was not the case. Methoxamine is known to be a directly acting sympathomimetic amine and its effects are not reduced by depletion of noradrenaline stores (Hoffman & Lefkowitz, 1990). It is therefore safe to conclude that a greater inotropic effect of methoxamine on myocardium of young than of adult rats was due to direct activation of α_1-adrenoceptors.

Ontogeny of the myocardium is a complex phenomenon involving the development of myocardial cells, blood vessels, interstitial cells and neural elements. Of these the development of neural elements is of particular significance with respect to sensitivity to autonomic drugs (Friedman et al., 1968; Friedman, 1972; Adolph, 1971; Hall 1957, Roeske and Wildenthal, 1981). Therefore attempt was made to modify the time-course of cardiac innervation and receptor ontogenesis to find out if these manipulations could modify the inotropic effects of methoxamine.

In order to investigate if the pattern of maturation of the α_1-mediated effect could be altered by neuronal or hormonal treatments, influence of chemical sympathectomy by 6-hydroxydopamine (6-OHDA) and the influence of injections of glucocorticoid and triiodothyronine to newborn rats on inotropic activity of methoxamine was studied. There are reports in the literature suggesting an increase in α_1-adrenoceptor density and sensitivity after chemical sympathectomy by 6-OHDA treatment (Yamada et al., 1984; Ishii et al., 1982). In the present study, short or long-term administration of 6-hydroxydopamine did not exert any significant effect on the inotropic response of the ventricles to methoxamine; however, the response to isoprenaline was potentiated. It would thus seem that sympathetic innervation might modify inotropic responses mediated by ß- but not α-receptors. This would seem logical if it is assumed that endogenous neurotransmitter does not function as a natural ligand for cardiac α-adrenoceptors.

Glucocorticoids and the thyroid hormone are important in cellular and neuronal growth (Lau and Slotkin, 1979; Kennedy and Ziegler, 1991; Slotkin et al., 1992). The present study determined if dexamethasone and triiodothyronine could perhaps enhance the maturation of the α_1-adrenoceptor-mediated functions. It was found that neither dexamethasone nor triiodothyronine altered the maturation pattern of myocardial inotropic responses to autonomic drugs. Our results differ form those of Lau and Slotkin (1981) who reported that dexamethasone treatment suppresses sympathetic cardiac responses in the neonatal rat but not in the adult rat. The same investigators have also reported that neonatal triiodothyronine treatment enhances the development of the noradrenergic synapse resulting in the premature appearance of functional sympathetic neurotransmission (Lau and Slotkin, 1979). On the other hand, our results are in conformity with those reported by other investigators (Benfey and Varma, 1963, Ishac et al., 1983) who found that the maximal inotropic effect to agonists is not altered by the administration of triiodothyronine.

As mentioned above, hormonal treatment or sympathectomy did not transform inotropic response to methoxamine on neonatal hearts to adult level. Studies were done to find out if the greater effect of methoxamine on neonatal than adult rat ventricles was due to a higher density or affinity of α_1-adrenoceptors in the myocardium of young than of adult animals. The working hypothesis was that the decrease in the α_1-adrenoceptor mediated effects in older animals could be due to an age-dependent decrease in the density and/or affinity of adrenoceptors and/or a decrease in the efficiency of the receptor-second messenger coupling.

We did not find any significant difference in the density and affinity of α_1- or β_1-adrenoceptors in ventricles from 1-week and 10-weeks old rats. Several workers have investigated age-dependent changes in myocardial adrenoceptors. A lack of age-dependent difference in α_1- and β_1-adrenoceptors found in this study is in conformity with data of some workers (Chen et al., 1979; Yamada et al., 1980; Roeske et al., 1979). Data of this study suggest that receptor concentration reaches adult level within a few days after birth in rats. In any case, a slight albeit significant change in receptor numbers does not necessarily imply change in response since only a small proportion of receptors are normally needed for a full expression of effects (Nickerson, 1956).

The age-dependent difference in the inotropic effect of methoxamine could be due to differences in receptor-response coupling. The second messenger for α_1-adrenoceptor is primarily inositol 1,4,5 triphosphate (IP_3). A greater methoxamine-induced increase in IP_3 in ventricles of 1 week old than of adult rats could explain the supersensitivity of the neonatal heart to α_1-adrenoceptor agonists. However, the results were quite the opposite. Although the basal IP_3 in the 1-week old rat ventricle was higher than in the 10-week old rat ventricle, methoxamine produced a concentration dependent increase in the IP_3 accumulation in the adult rat ventricle but

was without effect on the myocardium of 1 week-old rats.

The assay technique used in this study could measure accumulation of IP_3 and not the rate of its formation. It is thus possible that IP_3 was being degraded at a faster rate in the young than in the adult rat myocardium leading to false negative results. On the other hand, a significantly higher basal level of IP_3 in the heart of 1 week old than of adult rats would suggest that a faster degradation of IP_3 in neonatal heart is unlikely. This latter phenomenon that is a relatively high basal IP_3 in neonatal heart may itself be the reason that methoxamine failed to increase it further. However, methoxamine did increase the myocardial contractility over basal and hence would be expected to also increase IP_3 over basal regardless of the basal value.

In any case, results of this study fail to explain the underlying mechanism of the enhanced inotropic effect of methoxamine on ventricular preparations from rats up to 2 weeks of age. One possible explanation could be that in the young rat ventricle, the receptor is coupled to another second messenger such as cAMP. There are reports in the literature that in the adult heart protein kinase C is not involved in the α_1-adrenoceptor mediated inotropic effect (Endou et al., 1991). An alternate mechanism could be that probably in the young heart the α_1-adrenoceptor mediated inotropic effect is through the activation of diacylglycerol (DAG). Also, α_1-adrenoceptor agonists could directly act as calcium ionophores in hearts of young rats without involving the classical adrenoceptors. These possibilities need to be studied.

4.2. Conclusions

This study found that there is an age-dependent decrease in the α_1-adrenoceptor mediated inotropic effect in rats as revealed by responses to methoxamine and phenylephrine. On the other hand, there is no significant age-dependent change in the response to β_1-adrenoceptor agonists such as isoprenaline and noradrenaline. The work presented in this thesis failed to delineate the underlying mechanism of the enhanced inotropic effect of methoxamine and phenylephrine in ventricles of 0.5 to 2 week old rats, relative to adult rat ventricles. Treatments with 6-hydroxydopamine, dexamethasone and triiodothyronine did not alter the pattern of maturation of myocardial inotropic responses. The differences in the inotropic effects of methoxamine on neonatal and adult myocardium could not be attributed to difference in adrenoceptor density or generation of known second messenger for α_1-adrenoceptor, namely, inositol 1,4,5 triphosphate (IP_3). It is, however, possible that α_1-adrenoceptors recruit alternate second messenger in early life and maturation restores the coupling of these receptors to IP_3. This possibility needs to be studied.

SECTION 5: REFERENCES

Abel, J.J and Crawford, A.C. (1897): On the blood-pressure raising constituent of the suprarenal capsule. Johns Hopkins Hosp., 8:151-157.

Ablad, B., Carlsson, B., Dahloff, C. and Hultberg, E. (1974): Cardiac effects of ß-adrenergic receptor antagonists. Adv. Cardiol. 12:290-302.

Adolph, E.F. (1971): Ontogeny of heart rate controls in hamster, rat, and guinea pig. Am. J. Physiol. 220: 1896-1902.

Aghajanian, G.K. (1985): Modulation of a transient outward current in serotonergic neurones by α_1-adrenoceptors. Nature 315:501-503

Agrawal, O.K., Triggle, C.R. and Daniel E.E. (1984): Pharmacological characterization of the postsynaptic alpha adrenoceptors in vascular smooth muscle from canine and rat mesenteric vascular beds. J. Pharmacol. Exp. Ther. 229:831-838

Ahlquist, R.P. (1948): A study of the adrenotrophic receptors. Am. J. Physiol. 154:586-600.

Artman, M., Kithas, P.A., Wike, J.S. and Strada, S.J. (1988): Inotropic responses change during postnatal maturation in rabbit. Am. J. Physiol. 255: H335-H342.

Bacq, Z.M. (1937): Presence, dans l'humeur aqueuse du lapin, d'une substance sympathicomimetique apres stimulation du sympathique cervical. Compt. rend. soc. de. Biol. 107:1584-1586.

Baker, S.B., Boyd, H.M. and Potter, L.T. (1980): Distribution and function of ß-adrenoceptors in different chambers of the canine heart. Br. J. Pharmacol. 68:57-63.

Barger, G. and Dale, H.H. (1910): Chemical structure and sympathomimetic action of amines. J. Physiol (Lond). 41:19-51

Benfey, B.G. and Varma, D.R. (1963): Cardiac and vascular effects of sympathomimetic drugs after administration of triiodothyronine and reserpine. Br. J. Pharmacol. 21:174-181.

Benfey, B.G. and Varma, D.R. (1967): Interactions of sympathomimetic drugs, propranolol and phentolamine, on atrial refractory period and contractility. Br. J. Pharmacol. 30:603-611

Benfey, B.G., Varma, D.R. and Yue, T.L. (1983): Myocardial inotropic responses and adrenoceptors in protein-deficient rats. Br. J. Pharmacol. 80: 527-531.

Berridge, M.J. and Irvine, R.F. (1989): Inositol phosphates and cell signalling. Nature 341: 197-205.

Berthelson, S. and Pettinger, W.A. (1977): A functional basis for classification of alpha-adrenergic receptors. Life. Sci. 21:595-606.

Birnbaumer, L., Abramowitz, J. and Brown, A.M. (1990): Receptor-effector coupling by G proteins. Biochim. Biophys. Acta. 1031:163-224.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248-254.

Brodde, O.E. (1988): The functional importance of beta1 and beta2 adrenoceptors in the human heart. Am. J. Cadiol. 62:24C-29C.

Brown, G.L. and Gillespie, J.S. (1957): The output of sympathetic transmitter from the spleen of the cat. J. Physiol (lond). 138:81-102.

Burnstock, G. (1969): Evolution of autonomic innervation of the viscera and cardiovascular system in vertebrates. Pharmacol. Rev. 21:247-324.

Buxton, I.L.O. and Brunton, L.L. (1985): Action of the cardiac α_1-adrenergic receptor- activation of cyclic AMP degradation. J. Biol. Chem. 26:6733-6737.

Cannon, W.B. and Rosenbleuth, A. (1933): Studies on the conditions of activity in endocrine glands, sympathin E and sympathin I. Am. J. Physiol. 104: 557-574.

Cannon, W.B. and Uridil, J.E. (1921): Studies on the conditions of activity in endocrine glands. VIII. Some effects on the denervated heart of stimulating the nerves of the liver. Am. J. Physiol. 58:353-354.

Chemtob, S., Aranda, J.V. and Varma, D.R. (1989): Effects of prostaglandins on isolated internal carotid and basilar arteries of the newborn and adult pig. Pediatr. Rev. Commun. 4: 101-109.

Chemtob, S., Guest, I., Potvin, W. and Varma, D.R. (1991): Ontogeny of responses of rabbit aorta to atrial natriuretic factor and isoproterenol. Dev. Pharmacol. Ther. 16: 108-115.

Corr, P.B., Shayman, J.A., Kramer, J.B. and Kipnis, R.J. (1981): Increased α-adrenergic receptors in ischemic cat myocardium. J. Clin. Invest. 67:1232-1236.

Cottechia, S., Schwinn, D.A., Randall, R.R., Lefkowitz, R.J., Caron, M.G. and Kobilka, B.K. (1988): Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. Proc. Natl. Acad. Sci. USA. 85:7159-7163.

Cros, G.H., Chanez, P.O., Michel, A., Boucard, M. and Serrano, J.J. (1988). Post-natal evolution of rat cardiac beta-adrenoceptors. Life Sci. 43: 699-706.

Cubeddu, L., Barnes, E.M., Langer, S.Z. and Weiner, N. (1974): Release of norepineprine and dopamine-ß-hydroxylase by nerve stimulation. I. Role of neuronal and extraneuronal uptake and of alpha presynaptic receptors. J. Pharmacol. Exp. Ther. 190:431-450.

Dale, H.H. (1954): The beginnings and prospects of neurohumoral transmission. Pharmacol. Rev. 6:7-13.

Dixon, W.E. (1907): On the mode of action of drugs. The Med. Mag. 16: 454-457.

Dixon, R.A.F., Sigal, I.S., Rands, E., Register, R.B., Candelore, M.R., Blake, A.D. and Strader, C.D. (1987): Ligand binding to the ß-adrenergic receptor involves its rhodopsin-like core. Nature 326:73-77.

Dohlman, H.G., Bouvier, M., Benovic, J., Caron, M.G. and Lefkowitz, R.J. (1983). The multiple membrane spanning topography of the β_2-adrenergic receptors: localization of the sites of binding, glycosylation and regulatory phosphorylation by limited proteolysis. J. Biol. Chem. 262: 14282-14288.

Drew, G.M. and Whiting, S.B. (1979): Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle in vivo. Br. J. Pharmacol. 67:207-215.

Dubocovich, M.L. and Langer, S.Z. (1974): Negative feed-back regulation of noradrenaline release by nerve stimulation in the perfused cat's spleen: Differences in the potency of phenoxybenzamine in blocking the pre- and post-synaptic adrenergic receptors. J. Physiol (lond). 237:505-519.

Dubois-Reymond, E. (1877): Gesammette abhundlungen fur allegemeinen muskel- und nerven-physik. Two volumes. veit. u. comp., Leipzig.

Dukes, I.D. and Vaugham Williams, E.M. (1984): Effects of selective α_1-, α_2-, β_1- and β_2-adrenoceptor stimulation on potentials and contractions in the rabbit heart. J. Physiol (lond). 355:523-546.

Ehrlich, B.E. and Watras, J. (1988): Inositol 1,4,5-triphosphate activates a channel from smooth muscle sarcoplasmic reticulum. Nature 336:583-586.

Elliott, T.R. (1905): The action of adrenalin. J. Physiol (lond). 32:401-457.

Emirone, L.J., Marullo, S., Briend-Sutren, M.M., Patey, G., Tate, K., Deavier-Klutchko, C. and Strosberg, A.D. (1989): Molecular characterization of the human β_3-adrenergic receptor. Science 245:1118-1121.

Endoh, M. and Blinks, J.R. (1988): Actions of sympathomimetic amines on the calcium transients and contractions of rabbit myocardium: Reciprocal changes in myofibrillar responsiveness to calcium mediated through α- and β-adrenoceptors. Circ. Res. 62.247-265.

Endou, M., Hattori, Y., Tohse, N. and Kanno, M. (1991): Protein kinase C is not involved in α_1-adrenoceptor mediated positive inotropic effect. Am. J. Physiol. 260: H27-H36.

Fain, J.N. and Berridge, M.J. (1979): Relationship between hormonal activation of phosphatidylinositol hydrolysis, fluid secretion and calcium flux in the blowfly salivary gland. Biochem. J. 178:45-58.

Ferry, D.R. and Kaumann, A.J. (1987): Relationship between ß-adrenoceptors and calcium channels in human ventricular myocardium. Br. J. Pharmacol. 90:447-457

Freidman, W.F. (1972): The intrinsic physiologic properties of the developing heart. Prog. Cardiovasc. Dis. 15:87-111.

Friedman, W.F., Pool, P.E., Jacobwitz, D., Seagren, S.C. and Braunwald, E. (1968): Sympathetic innervation of the developing rabbit heart: biochemical and histochemical comparisons of fetal, neonatal, and adult myocardium. Cir. Res. 23: 25-32.

Gengo, P.J., Bowling, N., Wyss, V.L. and Hayes, J.S. (1988): Effects of prolonged phenylephrine infusion on cardiac adrenoceptors and calcium channels. J. Pharmacol. Exp. Ther. 244:100-105.

Gomez, H. (1958): The development of the innervation of the heart in the rat embryo. Anat. Rec. 130:53-71.

Govier, W.C., Mosal, N.C., Whittington, P. and Broom, A.H. (1966): Myocardial alpha and beta adrenergic receptors as demonstrated by atrial functional refractory-period changes. J. Pharmacol. Exp. Ther. 154:255-263.

Hall, E.K. (1957): Acetylcholine and epinephrine effects on the embryonic rat heart. J. Cell. Comp. Physiol. 49: 187-207.

Hanna, M.K. and Khairallah, Ph.A.(1986): Alterations of myocardial α_1-adrenergic receptors in hypertensive cardiac hypertrophy in the rat. Arch. Int. Pharmacodyn. 283:80-93.

Hausdorf, W.P., Caron, M.G. and Lefkowitz, R.J. (1990): Turning off the signal: desensitization of ß-adrenergic receptor function. FASEB. J. 4:2881-2889.

Hoffman, B.B. and Lefkowitz, R.J (1990): In, Catecholamines and sympathomimetic drugs. In, Goodman and Gilman's The pharmacological basis of therapeutics VIII edition (Gliman, A.G.; Rall, T.W.; Nies, A.S.and Taylor, P. eds.) pp188-190.

Ishac, E.J.N., Pennefather, J.N. and Handberg, G.M. (1983): Effect of changes in thyroid state on atrial α- and ß-adrenoceptors, adenylate cyclase activity, and catecholamine levels in the rat. J. Cardiovasc. Pharmacol. 5:396-405.

Ishii, K., Shigenobu, K. and Kasuya, Y. (1982): Postjunctional supersensitivity in young rat heart produced by immunological and chemical sympathectomy. J. Pharmacol. Exp. Ther. 220:209-215.

Iversen, L.L., Champlain, J.D., Glowinski, J. and Axelrod, J. (1967): Uptake, storage and metabolism of norepinephrine in tissues of the developing rat. J. Pharmacol. Exp. Ther. 157: 509-516.

Johnson, R.D. and Minneman, K.P. (1986): Differentiation of α_1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. Molec. Pharmacol. 32:239-246.

Kennedy, B. and Ziegler, M.G. (1991): Cardiac epinephrine synthesis: regulation by a glucocorticoid. Circulation 84: 891-895.

Klein, D.C., Sugden, D. and Weller, J.L. (1983): Postsynaptic α-adrenergic receptors potentiate the ß-adrenergic stimulation of pineal serotonin N-acetyltransferase. Proc. Natl. Acad. Sci. USA. 80: 599-603.

Kunos, G. and Mucci, L. and O'Regan, S. (1980): The influence of hormonal and neuronal factors on rat heart adrenoceptors. Br. J. Pharmacol. 71:371-386.

Kunos, G. and Nickerson, M. (1976): Temperature-induced interconversion of α- and ß-adrenoceptors in the frog heart. J. Physiol. (Lond) 256: 23-40.

Kunos, G. and Nickerson, M. (1977): Effects of sympathetic innervation and temperature on the properties of rat heart adrenoceptors. Br. J. Pharmacol. 59:603-614.

Kunos, G. and Nickerson, M. (1978): Thyroid control of rat and rabbit adrenoceptors. Fed. Proc. 37:684A.

Kunos, G., Vermes-Kunos, I. and Nickerson, M. (1974): Effects of thyroid state on adrenoceptor properties. Nature 250: 779-781.

Lands, A.M. Arnold, A., McAuliff, J.P., Luduena, F.P. and Brown, T.G. (1967): Differentiation of receptor systems activated by sympathomimetic amines. Nature 214: 597-598.

Langer, S.Z. (1970): The metabolism of[^{3}H]-noradrenaline released by electrical stimulation from the nictitating membrane of the cat and from the vas deferens of the rat. J. Physiol (lond). 208:515-546.

Langer, S.Z. (1981): Presynaptic regulation of the release of catecholamines. Pharmacol. Rev. 32:337-362.

Langley, J.N. (1905): On the reaction of cells and nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and curare. J. Physiol (lond). 33:374-400.

Lau, C. and Slotkin, T.A. (1979): Accelerated development of rat sympathetic neurotransmission caused by neonatal triiodothyronine administration. J. Pharmacol. Exp. Ther. 208: 485-490.

Lau, C. and Slotkin, T.A. (1980): Maturation of sympathetic neurotransmission in the rat heart.II. Enhanced development of presynaptic and postsynaptic components of noradrenergic synapses as a result of neonatal hyperthyroidism. J. Pharmacol. Exp. Ther. 212:126-130.

Lau, C. and Slotkin, T.A. (1981): Maturation of sympathomimetic neurotransmission in the rat he art. VII. Suppression of sympathomimetic responses by dexamethasone. J. Pharmacol. Exp. Ther. 216:6-11.

Ledda, F., Marchetti, P. and Mugelli, A. (1975): Studies on the positive inotropic effect of phenylephrine: A comparison with isoprenaline. Br. J. Pharmacol. 54:83-90.

Leeb-Lundberg, L.M.F., Dickinson, K.E.J., Heald, S.L., Wikberg, J.E.S., Hagan, P O., DeBnardis, J.F., Winn, M., Arendsen, D.L., Lefkowitz, R.J. and Caron, M.G. (1984): Photoaffinity labelling of mammalian α_1-adrenergic receptors - identification of the ligand binding subunit with a high affinity radioiodinated probe. J. Biol Chem. 259:2579-2587

Lefkowitz, R.J. and Caron, M.G. (1988): Adrenergic receptor: model for the study of receptor coupled to guanine nucleotide regulatory proteins J. Biol. Chem. 263: 4993-4996.

Lefkowitz, R.J., Kobilka, B.K. and Caron, M.G. (1989): The new biology of drug receptors Biochem. Pharmacol. 38:2941-2948.

Lefkowitz, R.J., Stadel, J.M and Caron, M.G. (1983)· Adenylate cyclase coupled beta-adrenergic receptors. Ann. Rev. Biochem. 52: 159-186, 1983.

Limbird, L.E. (1988): Receptors linked to inhibition of adenylate cyclase: additional signalling mechanisms. FASEB J. 2:2686-2695.

Loewi, O. (1921): Uber humorale ubertragbarkeit der herzenwirkung. Pfluger. Arch. Ges Phys. 189:239.

Loewi, O. and Navratil, E. (1926): Uber humorale ubertragbarkeit der herznervenwirkung. X Mitteilung. Uber das schicksal des vagusstoff. Pfluger Arch Ges Phys 214 689

Mackenzie, E. and Standen, N.B. (1976)· Adrenergic innervation of the mouse heart revealed at an early stage using α-methylnoradrenaline. Cell. Tiss. Res. 173 129-132.

Mackenzie, E. and Standen, N.B. (1980): The postnatal development of adrenoceptor responses of isolated papillary muscles from rat. Pflugers Archiv 383: 185-187.

Majerus, P.W., Connolly, T.M., Deckmyn, H., Ross, T.S., Bross, T.E., Ishii, H., Bansal, V.S and Wilson, D.B. (1986): The metabolism of phosphoinositide-derived messenger molecules. Science 234:1519-1526.

Michell, R.H. (1975): Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta. 415:81-147.

Minneman, K.P. (1988): α_1-Adrenergic receptor subtypes, inositol phosphates, and sources of cell calcium. Pharmacol. Rev. 40:87-117.

Mugge, A., Reupcke, C. and Scholz, H. (1985): Increased myocardial α_1-adrenoceptor density in rats chronically treated with propranolol. Eur. J. Pharmacol. 112:249-252.

Mukherji, A., Haghani, Z., Brady, J., Bush, L., Mcbride, W., Buja, L.M. and Willerson, J.T. (1983): Differences in myocardial α- and ß-adrenergic receptor numbers in different species. Am. J. Physiol. 245:H957-H961.

Murphy, T.V. and Majewski, H. (1991): Prejunctional α_1-adrenoceptors in the rat kidney. Br. J. Pharmacol. 104:288P.

Nakashima, M. and Hagino, Y. (1972): Evidence for the existence of alpha adrenergic receptor in the isolated rat atria. Jap. J. Pharmacol. 22:227-233.

Nichols, A.J. and Ruffolo, R.R. (1991): Functions mediated by α-adrenoceptors. Prog. Basic Clin. Pharmacol. 8: 115-179.

Nickerson, M. (1956): Receptor occupancy and tissue response. Nature 178: 697-698.

O'Dowd, B.F., Lefkowitz, R.J. and Caron, M.G. (1989): Structure of the adrenergic and related receptors. Ann. Rev. Neurosci. 12: 67-83.

Oliver, G. and Schaffer,E.A. (1895): The physiological effects of extracts from the suprarenal capsules. J. Phys (lond). 18:230-276.

Otani, H., Otani, H. and Das, D.K. (1988): α_1-Adrenoceptor mediated phosphoinositide breakdown and inotropic response in rat left ventricular papillary muscles. Circ. Res. 62:8-17.

Pappano, A.J. (1977): Ontogenic development of autonomic neuroeffector transmission and transmitter reactivity in embryonic and fetal hearts. Pharmacol. Rev. 29:3-33.

Pettinger, W.A. (1977): Unusual alpha adrenergic receptor potency of methyldopa metabolites on melanocyte function. J. Pharmacol. Exp. Ther. 201:622-626.

Potvin, W. and Varma, D.R. (1990): Refractoriness of the gravid rat uterus to tocolytic and biochemical effects of atrial natriuretic peptide. Br. J. Pharmacol. 100: 341-347.

Robkin, M., Shepard, T.H. and Baum, D. (1974): Autonomic drug effects on the heart rate of early rat embryos. Teratology 9: 35-44.

Robkin, M.A., Shepard, T.H. and Dyer, D.C. (1976): Autonomic receptors of the early rat embryo heart: growth and development. Proc. Soc. Exp. Biol. Med. 151: 799-803.

Roeske, W.R. and Wildenthal, K. (1981): Responsiveness to drugs and hormones in the murine model of cardiac ontogenesis. Pharmacol. Ther. 14:55-66.

Rosen, M.R., Hordof, A.J., Ilvento, J.P. and Danilo,Jr. P. (1977): Effects of adrenergic amines on electrophysiological properties and automaticity of neonatal and adult canine purkinje fibers. Circ. Res. 40:390-400.

Schiebler, T.H. and Heene, R. (1968): Demonstration of catecholamine in the rat heart during ontogenesis. Histochemie. 14:328-334.

Schimmel, R.J. (1976): Roles of alpha and beta adrenergic receptors in control of glucose oxidation in hamster epididymal adipocytes. Biochim. Biophys. Acta. 428:379-387.

Scholz, H., Bruckner, R., Mugge, A. and Reupcke, C. (1986): Myocardial alpha-adrenoceptors and positive inotropy. J. Cell. Cardiol. 18:79-87.

Scholz, J., Schaefer, B., Schmitz, W., Scholz, H., Steinfath, M., Martin, L., Schwabe, U. and Puurunen, J. (1988): Alpha-1 adrenoceptor-mediated positive inotropic effect and inositol triphosphate increase in mammalian heart. J. Pharmacol. Exp. Ther. 245:327-335.

Seidler, F.J. and Slotkin, T.A. (1979): Presynaptic and postsynaptic contributions to ontogeny of sympathetic control of heart rate in the preweanling rat. Br. J. Pharmacol. 65. 431-434.

Seiler, S.M., Arnold, A.J. and Stanton, H.C. (1987): Inhibitors of inositol-triphosphate-induced calcium release from isolated platelet membrane vesicles. Biochem. Pharmacol. 36:3331-3337.

Shah, A., Cohen, I.S. and Rosen, M.R. (1988): Stimulation of cardiac alpha receptors increases Na/K pump current and decreases g_k via a pertussis toxin-sensitive pathway. Biophys. J. 54:219-225.

Shah, J. and Pant, H.C. (1988): Potassium-channel blockers inhibit inositol triphosphate-induced calcium release in the microsomal fractions isolated from the rat brain. Biochem. J. 250:617-620.

Shigenobu, K., Tanaka, H. and Kasuya, Y (1988): Changes in sensitivity of rat heart to epinephrine and isoproterenol during pre- and postnatal development and its relation to sympathetic innervation. Dev. Pharmacol. Ther. 11:226-236.

Slotkin, T.A., Lappi, S.E., McCook, E.C., Tayyeb, M.I., Eylers, J.P. and Seidler, F.J. (1992): Glucocorticoids and the development of neuronal function. effects of prenatal dexamethasone exposure on central noradrenergic activity. Biol. Neonate 61: 326-336.

Snider, M.R. and Gerald, M.C. (1982): studies on the mechanism of (+)-amphetamine enhancement of neuromuscular transmission: muscle contraction, electrophysiological and biochemical results. J. Pharmacol. Exp. Ther. 221:14-21.

Standen, N.B. (1978): The postnatal development of adrenoceptor responses to agonists and electrical stimulation in rat isolated atria. Br. J. Pharmacol. 64: 83-89.

Starke, K., Montel, H. and Schumann, H.J. (1971): Influence of cocaine and phenoxybenzamine on noradrenaline uptake and release. N.S. Arch. Pharmak. 270:210-214.

Steinkraus, V., Nose, M., Scholz, H. and Thormahlen, K. (1989): Time course and extent of α_1-adrenoceptor density changes in rat heart after ß-adrenoceptor blockade. Br. J. Pharmacol. 96.441-449.

Strader, C.D., Candelore, M.R., Rands, E. and Dixon, R.A.F. (1987): ß-Adrenergic receptor subtype is an intrinsic property of the receptor gene product. Mol. Pharmacol. 32:179-183.

Streb, H., Bayerdorffer, E., Haase, W., Irvine, R.F. and Schulz, I. (1984): Effect of inositol-1,4,5 triphosphate on isolated subcellular fractions of rat pancreas. J. Memb. Biol. 81.241-253.

Suttapone, S., Worley, P.F , Baraban, J.M. and Snyder, S.H. (1988): Solubilization, purification, and characterization of an inositol triphosphate receptor. J. Biol. Chem. 263:1530-1534.

Thevenod, F., Dehlinger-Kremer, M., Kemmer, T.P., Christian, A.L., Potter, B.V.L. and Schulz, I. (1989): Characterization of inositol 1,4,5 triphosphate sensitive (IsCaP) and insensitive (IisCaP) nonmitochondrial calcium pools in the rat pancreatic acinar cells. J. Memb. Biol. 109:173-186.

Thomas, R.F., Holt, B.D., Schwinn, D.A. and Liggett, S.B. (1992): Long-term agonist exposure induces upregulation of $ß_3$-adrenergic receptor expression via multiple cAMP response elements. Proc. Natl. Acad. Sci. USA. 89:4490-4494.

Tsuji, K., Tsutsumi, S., Ogawa, K., Miyazaki, Y. and Satake, T. (1987): Cardiac-alpha-1 and beta adrenoceptors in rabbits: effects of dietary sodium and cholesterol Cardiovasc. Res. 21:39-44.

Tung, L.H., Rand, M.J. and Louis, W.J. (1987): Calcium antagonists inhibit positive chronotropic responses to α_1-adrenoceptor activation in rat isolated atria. Eur. J. Pharmacol. 133:177-184.

Varma, D.R. (1991): Functional link betwe n myocardial α_1- and β_1-adrenoceptors in neonatal rabbits. Drug. Dev. Res. 24:251-259.

Varma, D.R. and Yue, T.L. (1986): Adrenoceptor blocking properties of atropine-like agents anisodamine and anisodine on brain and cardiovascular tissues of rats Br. J. Pharmacol 87: 588-594.

von Euler, U.S. (1946): In, Catecholamines (Blaschko, H and Muscholl, E. eds.) Handbuch der experimentellen pharmacologie, vol.33. S nger-Verlag, Berlin, 1972. pp. 186-230

Wagner, J. and Brodde, O.-E. (1978): On the presence and distribution of α-adrenoceptors in the heart of various species. N. S. Arch. Pharmacol. 302:239-254.

Wald, M., Borda, E.S. and Sterin-Borda,L. (1988): α-Adrenergic supersensitivity and decreased number of α-adrenoceptors in heart from acute diabetic rats. Can. J. Physiol. Pharmacol. 66:1154-1160.

Wenzel, D.G. and Su, J.L. (1966): Interaction between sympathomimetic amines and blocking agents on the rat ventricular strip. Arch. Int Pharmacodyn. 160:379-389

Wilson, C. and Lincoln, C. (1984): ß-Adrenoceptor subtypes in human, rat, guinea pig, and rabbit atrium. J. Cardiovasc. Pharmacol. 6:1216-1221.

Yabuuchi, Y. (1977): The ß-adrenoceptor stimulant properties of OPC-2009 on guinea-pig isolated tracheal, right atrial and left atrial preparations. Br. J Pharmacol 61:513-521.

Yamada, S., Yamamura, H.I. and Roeske, W.R. (1980): Ontogeny of mammalian cardiac α_1-adrenergic receptors. Eur. J. Pharmacol. 68:217-221.

Yamada, S., Yamamura, H.I. and Roeske, W.R. (1980): Characterization of alpha-1 adrenergic receptors in the heart using [^{3}H]WB101: Effect of 6-hydroxydopamine treatment. J. Pharmacol. Exp. Ther. 215:176-185.

Yamada, S., Yamamura, H.I. and Roeske, W.R. (1980): Alterations in cardiac receptors following 6-hydroxydopamine treatment in rats. Molec. Pharmacol. 18:185-192.

Yamada, S., Ishima, T., Tomita, T., Hayashi, M., Okada, T. and Hayashim E. (1984): Alterations in cardiac alpha and beta adrenoceptors during the development of spontaneous hypertension. J. Pharmacol. Exp. Ther. 228:454-460.