Modern Developments in Cholinergic (Muscarinic) Receptors and Drugs

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Proceedings of a Symposium, organized by the Dutch Pharmacological Society in Oss, The Netherlands, on September 18, 1987

Edited by

P. A. van Zwieten and E. Schönbaum

55 figures and 18 tables

Gustav Fischer Verlag · Stuttgart · New York · 1989

Prof. Dr. P.A. van Zwieten
University of Amsterdam
Dept. of Pharmacotherapy
Meibergdreef 15
NL-1105 AZ Amsterdam Zuidoost
The Netherlands

Prof. Dr. E. Schönbaum
Organon Scientific Development Group
Dept. of Pharmacology
P.O. Box 20
5340 BH Oss, The Netherlands

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Modern developments in cholinergic (muscarinic) receptors and drugs : proceedings of a symposium in Oss, the Netherlands, on September 18, 1987 / organized by the Dutch Pharmacolog. Soc. Ed. by P.A. van Zwieten and E. Schönbaum. – Stuttgart ; New York : Fischer, 1989
(Progress in pharmacology and clinical pharmacology ; Vol. 7, No 1)
ISBN 3-437-11186-8
NE: Zwieten, Pieter A. van [Hrsg.]; Dutch Pharmacological Society; GT

Library of Congress Card-No. 88-30087

All business correspondence should be made with:
Gustav Fischer Verlag GmbH & Co. KG, Wollgrasweg 49, 7000 Stuttgart 70, Telefon (0711) 45 80 30, Bankkonto: Stuttgarter Bank Nr. 45290 (BLZ 60090100), Deutsche Bank 8020000 (BLZ 60070070), Commerzbank, 8799900 (BLZ 60040071), Postgiro Stuttgart Nr. 13556-709 (BLZ 60010070).

For USA and Canada:
VCH Publishers Inc., 303 N.W. 12th Avenue, Deerfield Beach, Florida 33442-1705, USA

Composed by: Typobauer Filmsatz GmbH, Scharnhausen
Printed and bound by: Gulde-Druck GmbH, Tübingen
Printed in Germany

ISBN 3-437-11186-8
US ISBN 0-89574-277-2
ISSN 0934-9545

Preface

The cholinergic system and the drugs interacting with it have been in a rather static phase for several years. Most pharmacologists and clinicians took it for granted, that the knowledge of this system did not greatly advance and that cholinergic and anticholinergic drugs were hardly the subject of relevant new developments. This situation has dramatically changed in the course of the past five years.

The major new finding in this field was that apparently muscarinic receptors should be subdivided in at least two and probably more than two types, with different affinities for agonists and antagonists. The recognition of different muscarinic (M)-receptor subtypes has greatly stimulated the search for new drugs with more selectivity for certain of these receptor subtypes. The M_1-receptor antagonist pirenzepine and the recently discovered cardioselective antagonist AF-DX 116 are examples of this development.

The particular interest in the muscarinic receptor field and its rapid development prompted the Dutch Pharmacological Society to devote its yearly symposium at Oss to this subject. Both fundamental and more applied aspects of the subject were discussed by experts in the field, thus presenting a fairly complete and most useful state of the present situation. The symposium was held on September 18, 1987, in the Dr. Saal van Zwanenberg Auditorium of Organon International BV, Oss, The Netherlands. The Dutch Pharmacological Society expresses its gratitude to Organon International for being a generous host.

The Society is also indebted to the following pharmaceutical companies:
CIBA-Geigy – Arnhem
Duphar – Weesp
Glaxo BV – Hoofddorp
Hoechst Pharma – Amsterdam
ICI Holland BV – Rotterdam
Parke-Davis – Amstelveen
Sandoz BV – Uden
whose financial support made the symposium possible.

The Editors of the present volume and the Society thank the Editorial Board of Progress in Pharmacology and the Publishers (Gustav Fischer Verlag) for allowing the publication of the symposium proceedings in its present form.

The competent organizational and secretarial assistance of Mrs. E. Zeeman is gratefully acknowledged.

The Editors.

Contents

Progress in Pharmacology and Clinical Pharmacology · Vol. 7/1
Gustav Fischer Verlag · Stuttgart · New York · 1989

Muscarinic Receptor Subtypes: Historical Development

R. Hammer

FL Biochemistry, Boehringer Ingelheim Zentrale GmbH, D-6507 Ingelheim, Germany

In reviewing the historical development of the concept of muscarinic receptor subtypes, one has to quote and to recognize two very early papers.

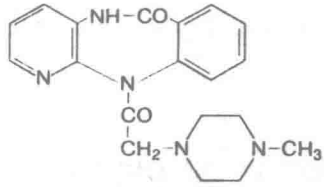
The first paper was published by Riker and Wescoe in 1951 (1). It described the unusual antimuscarinic profile of the neuromuscular blocking agent gallamine. For this nicotinic blocker antimuscarinic side effects on the heart were observed in doses well below those inhibiting other muscarinic functions. This was the first demonstration of organ selectivity in the muscarinic system. Today gallamine is classified as the prototype of an allosteric muscarinic receptor antagonist endowed with cardioselectivity.

The second paper dates back to 1961 (2). In this study Roskowsky described the selective muscarinic action of McN-A 343. This agent exerted a pronounced ganglionic stimulation blocked by low doses of atropine without appreciable effects on other muscarinic systems, in particular on muscarinic effector organs. Even today, more than 25 years later, the increase of arterial blood pressure in the pithed rat evoked by McN-A 343 has remained a crucial in vivo model in the search for selective muscarinic drugs.

Although both papers represented early suggestions of muscarinic receptor heterogeneity, and stimulated a great deal of related work in the following decades, it was not until the eighties that the existence of subtypes has been generally accepted.

The broad recognition of muscarinic receptor subtypes came with the discovery of new pharmacological tools from which three are particularly worth mentioning: pirenzepine (3), hexahydrosiladifenidol (4) and AF-DX 116 (5, 6). Each of these compounds is a pure muscarinic antagonist exhibiting a unique selectivity profile.

Fig. 1 shows the chemical structure of pirenzepine (PZ). It is a tricyclic compound with unusual hydrophilic properties (7). The lack of lipid solubility is explained mainly by the presence of the two acid amino groups (-N-CO-groups) which represent strong dipoles and therefore are responsible for the watersolubility of the drug. As a consequence, PZ does not penetrate the lipophilic blood-brain barrier to an appreciable extent. Its pharmacological actions are limited to peripheral organs.



PIRENZEPINE

Fig. 1: The chemical structure of pirenzepine

Nevertheless, the first indications that PZ might distinguish between different subclasses of muscarinic receptors stem from binding studies on subcellular preparations of the rat brain (8).
Fig. 2 shows binding curves of N-methyl-scopolamine (NMS), a classical antimuscar-

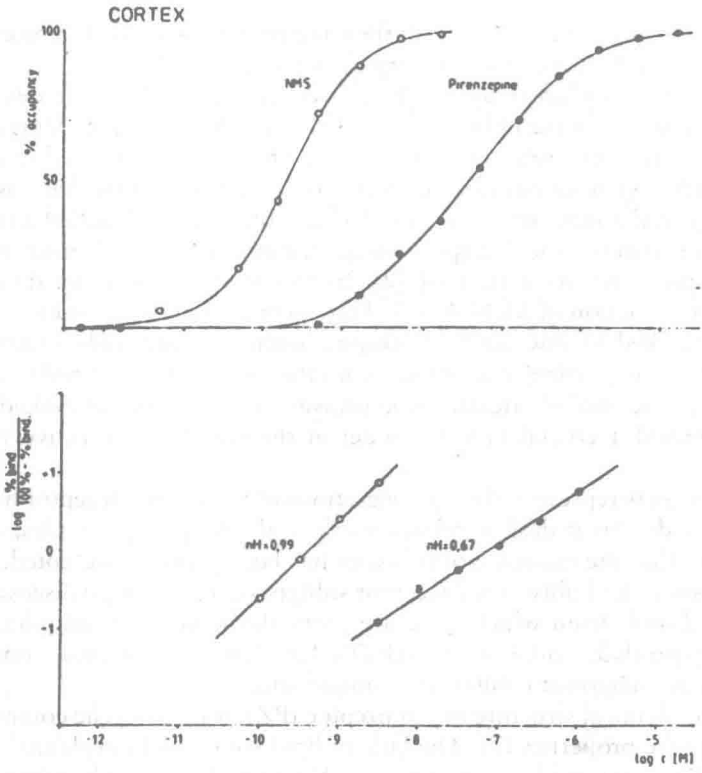


Fig. 2: Binding curves of N-methyl-scopolamine (NMS) and pirenzepine to muscarinic receptors of rat cerebral cortex. Data are derived from competition against ^{3}H-NMS. The lower panel shows the Hill-transformation. nH = Hill coefficient.

inic, and PZ to muscarinic receptors of the cerebral cortex. From these early data two important results could be derived.

1. PZ binds to central muscarinic receptors at submicromolar concentrations, i.e. in a pharmacologically relevant range, and
2. unlike NMS, PZ exhibits a flat binding curve with a Hill coefficient significantly less from unity. This is shown in the lower panel of the graph.

A simple explanation for such a behaviour is that PZ is able to distinguish between different subclasses of muscarinic receptors in the brain, a view supported by kinetic analysis of the experimental data.

Fig. 3 depicts the computer fit of the binding data in the cerebral cortex according to a 1-site and a 2-site model. It is obvious, that only the 2-site model adequately describes the experimental binding values. The analysis with the 1-site model leads to large and systematic deviations, thus rejecting the model assumptions.

According to the 2-site model, the rat cerebral cortex contains about 60% high affinity and 40% low affinity receptors for PZ, with dissociation constants of 10–20 nM and 200–400 nM, respectively.

These heterogenous binding curves of PZ were exceptional, since such a behaviour had not been shown before for any other antimuscarinic agent. At that time the Burgen group in London had already investigated a great variety of muscarinic antagonists, but in all cases binding curves were compatible with the existence of a single receptor

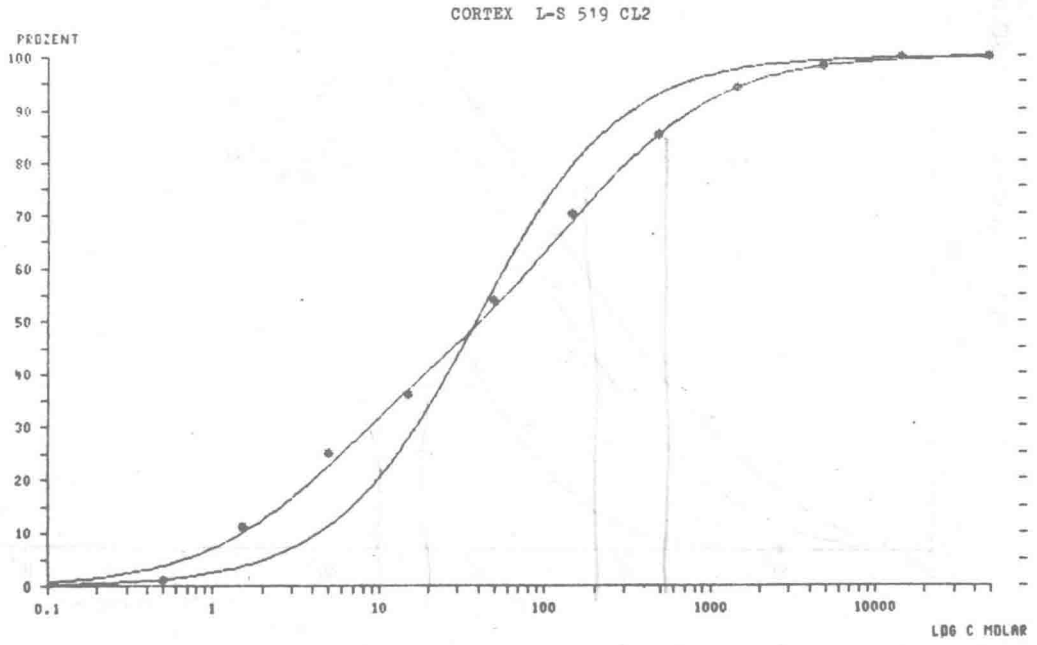


Fig. 3: Best fit of the experimental binding values of PZ in rat cerebral cortex according to a 1-site model (steep curve) and a 2-site model (flat curve).

population in the brain (9). Different results, however, had been obtained by the Burgen group with agonists. Muscarinic agonists, like acetylcholine, carbachol, oxotremorine and others exhibited also heterogenous binding in brain preparations (10). Therefore the question arose whether the antagonist PZ behaved like an agonist. This was easy to test, since binding studies in different brain regions had revealed a distinct affinity pattern for muscarinic agonists. Brain areas like the cerebellum and medulla-pons contained almost exclusively high affinity binding sites for agonists. In the cerebral cortex similar proportions of high and low affinity sites were present. In other regions, like hippocampus, low affinity sites for agonists prevailed.

The next step, therefore, was to investigate the binding of PZ in particular characteristic brain areas, i.e. medulla-pons, cerebral cortex and hippocampus (Fig. 4). The results were surprising. Whilst agonists bind with low affinity in hippocampus, PZ occupancy occured in comparatively low concentrations suggesting the presence of a major proportion of high affinity sites in this area. The opposite holds true for the medulla-pons. Whilst this region contains primarily high affinity sites for agonists, PZ binds to a virtually homogenous population of low affinity receptors.

From these data it became clear that the antagonist PZ exhibited a reversed selectivity profile as compared to muscarinic agonists. This indeed was a strong argument for the existence of two independent muscarinic receptor subtypes in the CNS.

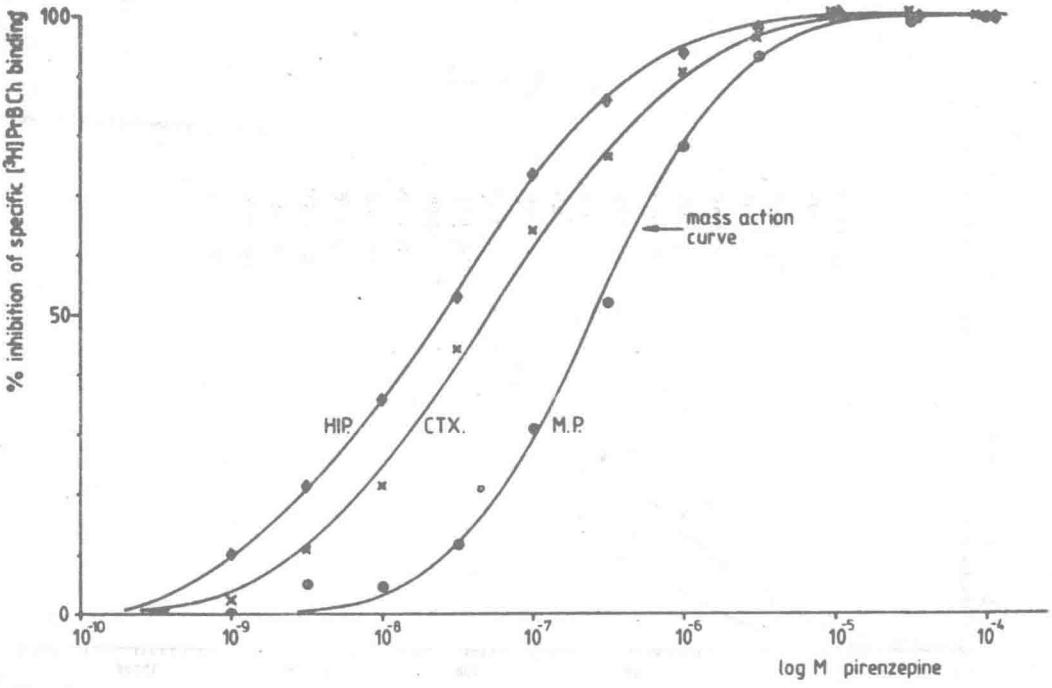


Fig. 4: Binding curves of PZ to muscarinic receptors of hippocampus, cerebral cortex and medulla-pons.

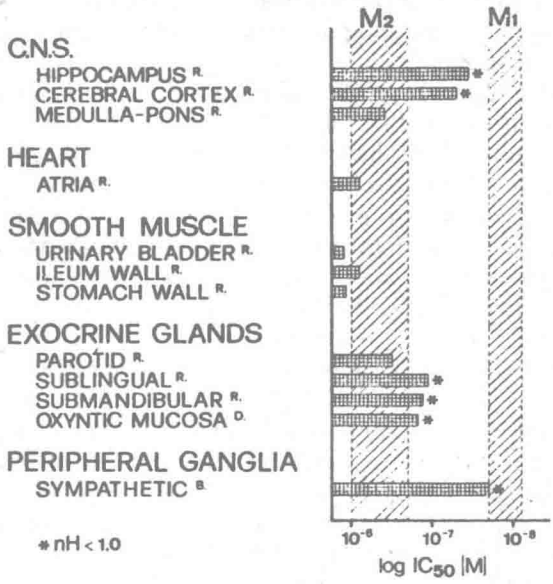


Fig. 5: Affinity profile of PZ to muscarinic receptors in various tissues (3). The IC50 value represents the concentration of PZ at which half saturation is reached in a given tissue. The asterisk indicates the presence of a heterogenous receptor population (R = rat, D = dog, B = bovine).

These findings of two subtypes of central muscarinic receptors were confirmed subsequently using direct binding studies with PZ (11) and autoradiography (15). According to a nomenclature independently proposed by Woodruff and Walker (12) and Goyal and Rattan (13) on the basis of results with McN-A 343, they were named M_1- and M_2-receptors.

Also in peripheral organs both high and low affinity receptors for PZ were detected. Fig. 5 summarizes affinity measurements with PZ in twelve characteristic tissues known to be controlled by muscarinic receptors (3, 14). Non-linear regression analysis of the PZ binding curves in these twelve body regions revealed a distinct distribution pattern of M_1- and M_2-receptors. Virtually homogenous populations of M_2-receptors were found in a variety of tissues, i.e. the medulla-pons, the heart, in different smooth muscle organs, and in the parotid gland. In some exocrine glands, i.e. sublingual gland, submandibular gland, and oxyntic mucosa, small proportions of M_1-receptors were detected (15–30%). A preponderance of M_1-receptors was observed in certain neuronal tissues, namely cerebral cortex, hippocampus, and sympathetic ganglia (55–75%).

Initially the existence of PZ-distinguishable subtypes has been demonstrated purely on the basis of binding results. In fact, binding studies may be considered as a very accurate technique for determining the affinity for antagonists. On the other hand,

they don't provide information on the functional properties of a receptor. Therefore a pharmacologically sound and meaningful subclassification has to be supported and confirmed by functional studies.

Table 1 shows a direct comparison of PZ-affinity estimates from in vitro pharmacological studies in isolated tissues (16, 17, 18, 19, 20) and from binding studies (3). Three representative muscarinic systems have been investigated: the atria, the ileum and sympathetic ganglia. It is evident that both methods clearly demonstrate the discriminatory power of PZ. As well in vitro pharmacology as binding techniques define muscarinic receptors in the atria and in the ileum as the low affinity M_2-subtype, whilst in sympathetic ganglia both methods reveal the presence of the high affinity M_1-subtype. With both techniques the difference in the affinity estimates amounts to a factor of about 40 (i.e. about 1,6 log units). Thus the concept of muscarinic receptor heterogeneity as revealed by PZ was fully supported by functional studies.

Although the subclassification of muscarinic receptors on the basis of PZ was confirmed by various groups (15), it soon became clear that the simple M_1/M_2-scheme was too simple to account for all the experimental observations of muscarinic receptor heterogeneity. With the advent of novel tools like hexahydrosiladifenidol (4) and AF-DX 116 (5, 6) evidence accumulated that M_2-receptors are heterogenous.

Fig. 6 shows the chemical structure of AF-DX 116 (21) which is the prototype of a class of competitive cardioselective antagonists. AF-DX 116 has the same tricyclic ring as

ESTIMATES OF PIRENZEPINE – AFFINITIES FOR FUNCTIONAL MUSCARINIC RECEPTORS IN ISOLATED TISSUES (pA_2 - VALUES) AND FROM BINDING STUDIES (- LOG IC 50 COR-VALUES)

	ATRIA	ILEUM	SYMP. GANGLIA	
M.E. PARSONS et al. 1979	–	6.50	–	IN VITRO PHARMACOLOGY
D.A. BROWN et al. 1980	–	6.99	8.36	IN VITRO PHARMACOLOGY
R.B. BARLOW et al. 1981	6.20	6.65	–	IN VITRO PHARMACOLOGY
M.P. CAULFIED et al. 1981	6.71	6.70	8.10	IN VITRO PHARMACOLOGY
H. FUDER et al. 1981	6.59	–	–	IN VITRO PHARMACOLOGY
R. HAMMER et al. 1980	6.08	6.10	7.70	BINDING

Table 1: Estimates of PZ-affinities for functional muscarinic receptors in isolated tissues (pA_2-values) and from ligand binding studies (- log IC50 values)

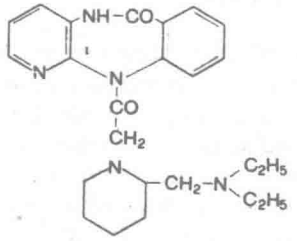


Fig. 6: The chemical structure of AF-DX 116

AF-DX 116

PZ, but a quite different side chain suggesting that it is the side chain which is primarily responsible for organ selectivity.

Fig. 7 shows binding curves of the compound in membranes of some typical tissues: the heart, the cerebellum, cerebral cortex, the submandibular and lacrimal gland (5). The compound exhibits large affinity variations in both peripheral organs and different regions of the brain.

From non-linear regression analysis of the binding curves in various peripheral and central tissues, all the data can be explained by the existence of three different subtypes

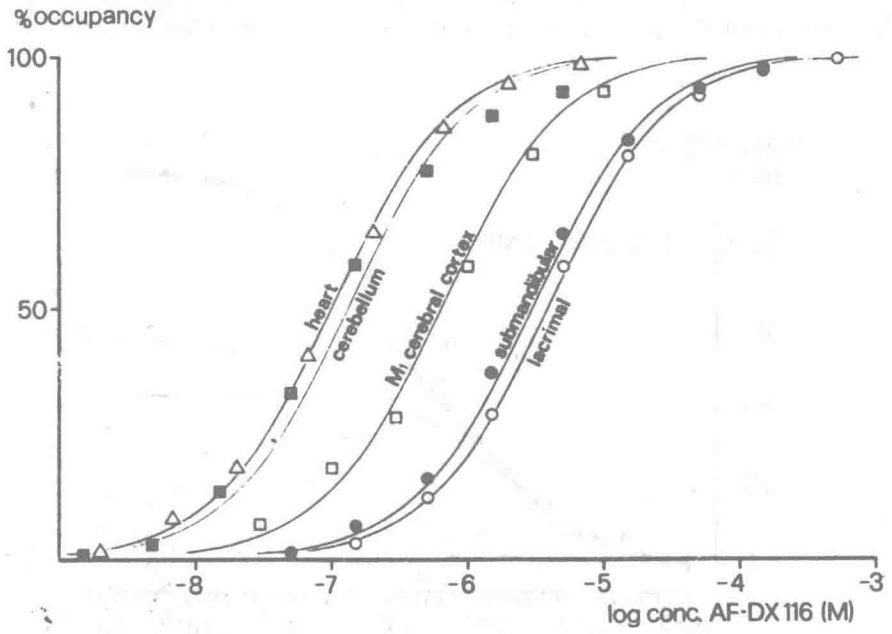


Fig. 7: Occupancy concentration curves of AF-DX 116 to muscarinic receptors in various peripheral and brain tissues (5) measured in competition against ^{3}H-PZ, (M_1-cerebral cortex) and ^{3}H-NMS (other tissues).

characterized by different affinities for AF-DX 116. These three subtypes are the M_1-receptor, the M_2-receptor of the cardiac type, and the M_2-receptor of the glandular type. Typical examples for these three subtypes are shown in Fig. 7.

The cardiac type is characterized by high affinity for AF-DX 116. It is found in various regions of the heart and in the CNS. Most receptors in the cerebellum and medulla-pons belong to the cardiac type. Its interaction with AF-DX 116 is characterized by a dissociation constant of about 10^{-7} M. The M_1-receptor exhibits intermediate affinity for AF-DX 116. In Fig. 7 the binding of AF-DX 116 to the M_1-receptor of the cerebral cortex is shown measured in competition against a low concentration of ^{3}H-PZ. The dissociation constant of AF-DX 116 for M_1-receptors amounts to about 7×10^{-7} M. Finally, the glandular type found in various exocrine glands has low affinity for AF-DX 116 with a dissociation constant of about 4×10^{-6} M. In Fig. 7 the binding curves to the submandibular and lacrimal glands are depicted. This low affinity subtype for AF-DX 116 is, however, not only found in glands, but also in certain regions of the brain. Fig. 8 shows the binding of AF-DX 116 to muscarinic receptors of the hypothalamus. This brain region is of particular interest, since AF-DX 116 exhibits a very flat binding curve in this tissue. It is evident, that the curve extends over a range of 5 log units. In accordance with this a Hill coefficient of approximately 0.6 can be calculated (22). This behaviour is in agreement with the view that both the cardiac and glandular type is present in this brain area. According to the computer fit about 40% of the receptors belong to the cardiac type and 60% to the glandular type, respectively.

It is important that the novel selectivity profile of AF-DX 116 is not limited to in vitro

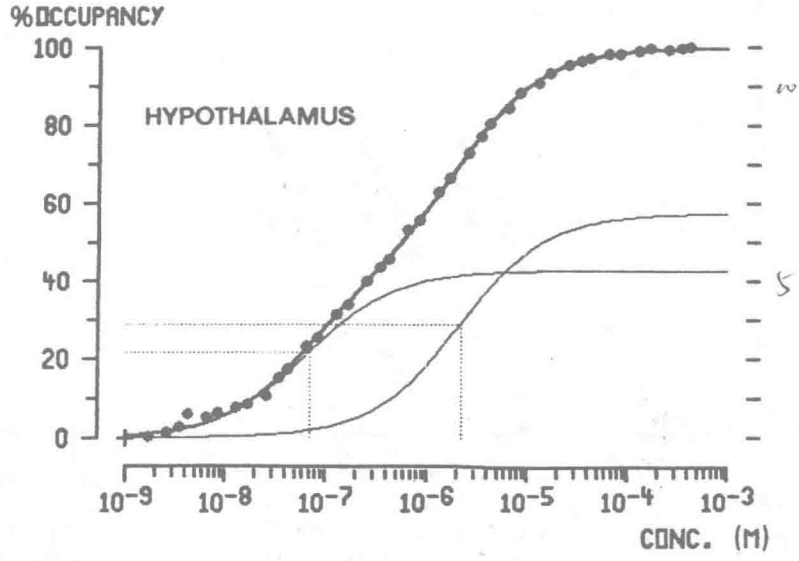


Fig. 8: Non-linear regression analysis of the occupancy concentration curve of AF-DX 116 to muscarinic receptors of the hypothalamus. Best fit according to a 2-binding site model (22).

binding studies. It can be demonstrated also in in vivo binding studies (23) and in functional in vitro and in vivo tests (6).

Fig. 9 shows a study in which four different muscarinic effects were investigated in the anaesthetized cat. All four effects were elicited by exogenous bethanechol: reduction in heart rate, increase in salivation, augmentation of urinary bladder tone and decrease in blood pressure. Atropine is a potent inhibitor of all four responses. However, it is not selective, being almost equiactive in all the investigated systems. AF-DX 116 behaves clearly different. It also inhibits dose dependently the four effects, but it demonstrates a clear selectivity in favour of the cardiac response. The selectivity is so pronounced, that in this model no overlap of the dose response curves between the heart and the other organs could be detected.

In the meantime AF-DX 116 is being investigated clinically (24). It ist the first

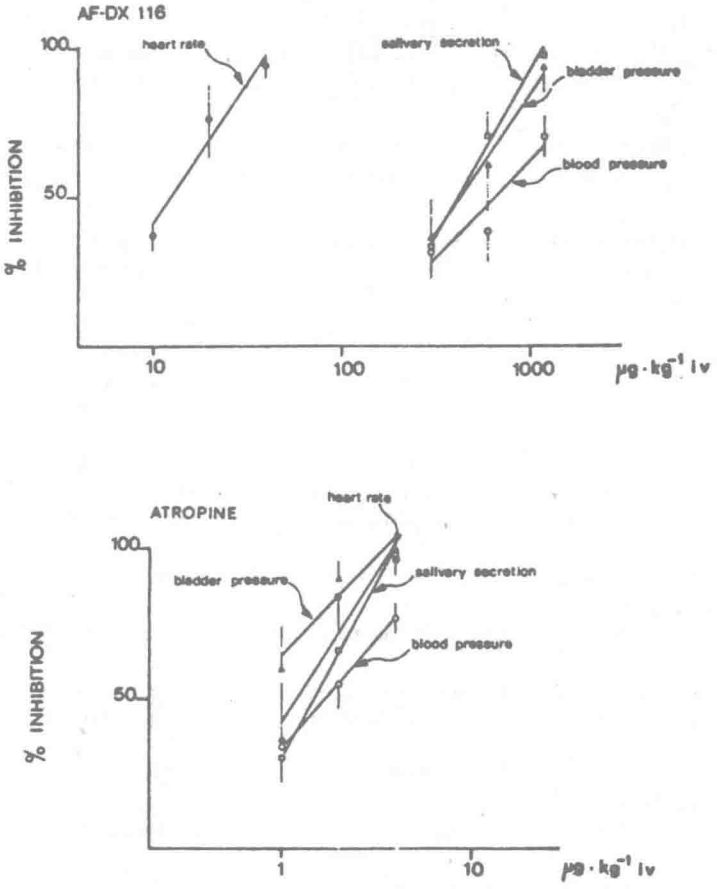


Fig. 9: Antagonism by AF-DX 116 (upper panel) and atropine (lower panel) of muscarinic responses elicited in the anaesthetized cat by bethanechol (6).

muscarinic antagonist which raises heart rate without inhibiting salivation thus confirming the preclinical results.
Recently, definitive evidence of the existence of distinct muscarinic receptor subtypes has been provided. Using molecular cloning techniques it was established that M_1-receptors from cerebral cortex and M_2-receptors from atria are indeed different polypeptides encorded by distinct genes (25, 26). Moreover, there are at least two further muscarinic receptor proteins with thus far unexplored pharmacological specificity (27).

Conclusions

With the availability of new pharmacological tools in the late 70-ies and in the 80-ies three different subtypes of the muscarinic receptor were identified:
the M_1-receptor (high affinity for pirenzepine-like compounds),
the M_2-receptor of the cardiac type (high affinity for AF-DX 116-like compounds) and
the M_2-receptor of the glandular/smooth muscle type (high affinity for hexahydrosiladifenidol and related agents).
Each of these subtypes is found in the periphery and in the CNS. Their existence has been demonstrated both by receptor binding and functional studies in animals and in man.
At present cloning experiments by several groups are shedding light into the molecular differences of these pharmacologically distinguishable muscarinic receptor subtypes.

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