Selective muscarinic receptor antagonists

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Subtypes of muscarinic receptors, first hypothesized by Birdsell et al. to explain the anomalous binding of cholinergic agonists to brain receptors, were later demonstrated with the selective muscarinic antagonist, pirenzepine⁵. Interestingly, the search for selective antagonists, which usually follows the acceptance of a concept, antedated by many years the notion of heterogeneity of muscarinic receptors. Intuitively scientists, realizing that muscarinic receptors subserve an extraordinary variety of biological functions, sought drugs which could influence these functions in a discrete fashion.

Since peripheral muscarinic receptors control gastric secretion and smooth muscle contraction, the two main objectives have been to find selective antiserotonergic compounds for use as antiulcer drugs and selective smooth muscle agents for use as antispasmodics. For this purpose, thousands of compounds have been synthesized during the last 30 years but only recently, with the introduction of pirenzepine into human therapy, has the goal of a selective anti-muscarinic agent been achieved.

The diverse and sophisticated investigational techniques available today enable demonstration of selective antimuscarinic action at different methodological levels. To show organ selectivity in vivo in animals and/or in man, is not sufficient to claim receptor selectivity for a new drug. In vitro studies, covering both functional aspects in isolated tissues and receptor binding to membrane fragments, are needed to complement in vivo investigations. Since all these experimental procedures, due to the inherent difference in biological complexities, have their specific advantages and limitations, we feel that, in order to prove receptor selectivity, a consistent picture should emerge from all these models.

In this paper we will discuss the evidence for a selective antimuscarinic action of two well-established agents, pirenzepine and gallamine. In addition, other compounds for which a receptor selective behaviour has been postulated will be mentioned.

Pirenzepine

Pirenzepine is a tricyclic compound (Fig. 1) which, unlike psychotropic tricyclic agents, penetrates poorly through the blood-brain barrier because of its pronounced hydrophobicity. Both experimental and clinical evidence attest to the selectivity of pirenzepine for the receptors governing gastric secretion. In man, significant reduction of gastric acid and pepsinogen secretion is obtained at plasma concentrations at which other antimuscarinic effects, like mydriasis, inhibition of gastric emptying, and impairment of oesophageal motility, do not occur. Tachycardia, which is common with other antimuscarinics after i.v. administration, is not observed with pirenzepine, thus allowing its parenteral use. Therapeutically, the drug has a wide margin of safety with no clinical contraindications.

Receptor binding studies have provided the first indication of the ability of pirenzepine to discriminate between muscarinic receptors in different tissues. Represented in Fig. 2 are the occupancy-concentration curves for pirenzepine to muscarinic receptors from membranes of various tissues, as determined in competition experiments against the radioligand N-methylscopolamine.

Immediately evident are the striking differences in affinity among tissues, measurable by the relative position of the curve on the abscissa. The inhibitory constants (Ki) express these differences in quantitative terms. Thus, the lowest Ki, found in autonomic ganglia (20 nM) reflects the extraordinary affinity of pirenzepine for the neural muscarinic receptors of this tissue, while the high value found in smooth muscle and heart (about 800 nM) represents the lowest point in affinity. In contrast, the classical antimuscarinic drugs, like atropine or N-methylscopolamine, when examined by means of binding techniques, generate superimposable binding curves and virtually the same Ki values for all tissues.

The 40-fold difference of Kᵢ, among various tissues is by no means the only difference between classical antimuscarinic agents and pirenzepine. An addi-
tional characteristic feature is represented by the unusual flat binding curves, observed in some organs, which do not obey simple mass action rules. This is expressed by Hill-coefficients significantly less than 1.0 (see Fig. 2) and signifies that pirenzepine interacts with muscarinic receptors which are heterogeneous within a given organ.

In fact, all binding data in various tissues are compatible with the presence of high affinity receptors characterized by a dissociation constant in the 10–20 nM range and with receptors having low affinity for pirenzepine with dissociation constants in the 200–800 nM range. Interestingly, the selective binding behaviour of pirenzepine which originally had been demonstrated indirectly, in competition with various muscarinic ligands, had recently been confirmed by direct binding, using [3H]pirenzepine as a radioligand.

The discriminatory properties of the drug have also been demonstrated in functional tests in vitro. Thus, pirenzepine displays a high affinity antagonism towards the muscarine-induced depolarization of isolated sympathetic ganglia (pA2 = 8.3) while it shows a low affinity antagonism (pA2 = 6.5–7.0) for the cholinergic induced contraction of the ileum and in the slowing of heart rate.

The use of a novel research tool frequently allows new insight into the physiology of organ functions. Recently, we have studied the antiserotonin action of pirenzepine in the isolated mouse stomach, an in vitro preparation in which acid secretion can be stimulated either by exogenous muscarinic agonists, like bethanechol, or by electrical field stimulation which mimics vagal excitation. The involvement of muscarinic receptors in the secretion evoked by both stimuli is demonstrated by the sensitivity to atropine or pirenzepine but, interestingly, the two antimuscarinic agents exhibit a differential behaviour in antagonizing exogenous or endogenous stimuli. When secretion is elicited by exogenous bethanechol, pirenzepine has weak inhibitory properties relative to atropine (approximately 30 times less potent). However, when vagal activation is the main determinant of secretion, pirenzepine exerts potent inhibitory activity and is only slightly less effective than atropine. The clinical correlates of these in vitro observations are the data showing the effectiveness of pirenzepine in inhibiting the acid secretion evoked in man by reflex activation of the vagus (sham feeding, fundic distension, etc.).

These findings are compatible with the notion that acid secretion can be evoked experimentally by two distinct muscarinic mechanisms. Firstly, as shown by several workers, exogenous agonists stimulate parietal cell receptors directly. Secondly, endogenous acetylcholine released through vagal stimulation induces acid secretion by activating muscarinic receptors remote from the effectors cells, presumably localized at the level of gastric ganglion cells. We postulate that pirenzepine affects gastric secretion by interacting with the high affinity muscarinic receptors of these latter cells.

The discriminatory properties of pirenzepine have been instrumental in the subclassification and nomenclature of muscarinic receptors. Recently, it was discovered that McN-A-343, a selective muscarinic agonist, and pirenzepine act on a common muscarinic receptor subtype. Thus, in functional terms, muscarinic receptors excitable by McN-A-343 and sensitive to pirenzepine are termed M1-receptors, as originally proposed by workers investigating the neural control of gastro-intestinal motility. Whereas the M1-receptor subtype prevails in neural tissues (autonomic ganglia, discrete brain areas), the muscarinic receptors with low affinity for pirenzepine, found mainly in peripheral effector organs, are termed M2-receptors.

**Gallamine**

Gallamine is a quaternary neuromuscular blocking drug used clinically as a muscle relaxant (Fig. 3). Like other neuromuscular blockers, gallamine is not a pure nicotinic receptor antagonist but interacts with muscarinic receptors as well. For example, the tachycardia noted in man concurrently with gallamine induced muscle relaxation, may be viewed as an antimuscarinic effect. Its antagonism of the negative chronotropic and inotropic effects exerted by muscarinic agonists in isolated atrial preparations supports this interpretation. Because these cardiac effects are observed at doses below those inhibiting muscarinic functions in gastro-intestinal and bladder smooth muscle or in salivary glands, gallamine is thought to possess a cardioselective antimuscarinic profile.

The interaction of gallamine with muscarinic receptors when examined in vitro on isolated atria is of the non-competitive type. Although it produces parallel rightward shifts of the log-dose–response curve to the agonists without depressing the maximum response, the magnitude of the shifts (dose–ratios) is not linear with drug concentrations, as would be expected for a simple competitive antagonism. At higher concentrations the dose ratios tend to a limiting value resulting in a flattening of the Schild-plot (Fig. 4).

Recently, the nature of the interaction of gallamine with muscarinic receptors was reinvestigated by studying its influence on the binding of muscarinic agonists and antagonists to cardiac receptors. As expected, gallamine displaced the binding curve of muscarinic ligands. However, the extent of the displacement did not vary linearly with increasing gallamine concentrations but approached a maximal value. The maximal shift differed for each agonist and antagonist examined, and was dependent on the tissue investigated. In agreement with the pharmacological data, the inhibitory effects of gallamine on the binding of muscarinic agents were much greater in the heart than in other tissues thus confirming its cardioselective action.

In conclusion, the data from both functional and binding studies are compatible with a model in which gallamine binds to a site which is not identical with the conventional acetylcholine binding site but allosterically modulates, in a negative co-operative fashion, the binding of agonists and antagonists to the conventional site.

It is possible that in the case of agon-
ists, such an allosteric interaction changes not only the affinity but also the efficacy; therefore, both binding studies and functional tests are necessary to fully characterize the effects. Finally, it should be mentioned that drugs which exhibit a negative co-operative effect greater than gallamine may falsely be identified as competitive antagonists because their effects are difficult to distinguish from competitive behaviour.

Secoverine

In-vivo investigations have shown secoverine to be nearly as potent as atropine in inhibiting contractions of the intestinal smooth muscle elicited by carbachol. Contrasting this strong inhibitory activity observed in other organ functions controlled by muscarinic receptors, such as pupil size, gastric emptying, voiding of the bladder, cholinergically induced secretion of various exocrine glands and heart rate. The only notable exception was the oxotremorine-induced central action which secoverine effectively attenuated. This in-vivo pharmacological profile, indicating a propensity to inhibit cholinergically-induced motor function lead to the conclusion that secoverine interacts with only one subclass of muscarinic receptors.

These properties which may contribute to its in-vivo smooth-muscle relaxing action, make the antimuscarinic mechanism of secoverine less clear cut.

So far, the demonstration of in-vitro receptor selectivity by use of binding techniques or isolated tissue preparations is lacking. To our knowledge, in-vitro studies in isolated gastro-intestinal smooth muscle and myometrium have indeed demonstrated potent antimuscarinic properties for secoverine (pA2 near 9.0) but affinity estimates for muscarinic receptors from tissues which do not respond in-vivo, like heart and salivary glands, have not been reported.

Such studies, which may minimize the contribution of non-muscarinic mechanisms and exclude complications through in-vivo metabolism, can definitely show whether the observed organ-specific action of secoverine in-vivo reflects selective antimuscarinic properties.

References


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