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Binding studies on two functional cardioselective antimuscarinic compounds

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Abstract—Vecuronium and himbacine are antimuscarinic compounds which in functional studies exhibited a ca 6- and 10-fold higher potency at cardiac muscarininc receptors than at ileal muscarininc receptors. However in binding studies both compounds failed to differentiate between [^{3}H](−)-QNB binding sites in guinea-pig atrial and ileal muscle homogenates. In the latter experiments, the dissociation constants of vecuronium in atria and ileum and that of himbacine in ileum were lower than the values determined functionally. The basis for the lack of cardioselectivity in binding studies is not known. These compounds add to the list of functional cardioselective muscarinic receptor antagonists that failed to display selectivity in binding studies with [^{3}H](−)-QNB.

Himbacine exhibited competitive cardioselective antimuscarinic activity in functional experiments having ca 10-fold higher affinity for cardiac than for ileal muscarinic receptors (Gilani & Cobbin 1986).

Vecuronium, a neuromuscular blocking drug with low potency for blocking cardiac muscarinic receptors has also been reported to exhibit some degree of cardioselectivity (Marshall et al 1980).

It was of interest to test the ability of these compounds to displace quinuclidinyl benzilate ([^{3}H](−)-QNB) in binding studies given that other functional cardioselective antagonists such as gallamine and pancuronium failed to differentiate between atrial and ileal muscarinic receptors in binding studies (Choo et al 1985; Nedoma et al 1985).

Methods

Binding experiments with [^{3}H](−)-QNB (40 to 80 pM) were conducted and analysed as previously described (Choo et al 1985) using 150 μg protein in atria or 50 μg protein in ileal longitudinal muscle.

Isolated tissue experiments were also conducted to establish the functional cardioselectivity of these compounds. The negative inotropic response to cholinomimetics in atria and contractile responses in ileal longitudinal muscle were used as a measure of cardiac and ileal muscarinic receptor stimulation, respectively.

Results

Both himbacine and vecuronium displaced [^{3}H](−)-QNB from a single site in the two tissues (Table 1). With each antagonist the K_D values were similar in the two tissues and there was no evidence of cardioselectivity.

Functional studies with the antagonist confirmed (Table 1) that both drugs had a cardioselective action as previously described (Marshall et al 1980; Gilani & Cobbin 1986). For himbacine the slope of the Arunlakshana-Schild (A-S) plot was linear in both atria and ileal longitudinal muscle. For vecuronium the slope was less than 1·0 in atria (0·86 ± 0·06, $P < 0·05$). In ileal smooth muscle only one concentration of vecuronium could be investigated due to its low potency and low solubility.

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Table 1. Comparison of the log dissociation constants from functional and binding experiments in guinea-pig tissues.

	-log dissociation constants ± s.e.m. (n)			
	Vecuronium		Himbacine	
	Atria	Ileum	Atria	Ileum
Functional study	5·2* ±0·03 (3)	4·4* ±0·1 (4)	8·4 ±0·1 (4)	7·4 ±0·2 (3)
[^{3}H](−)-QNB binding	6·1 ±0·01 (4)	6·2 ±0·01 (4)	8·0 ±0·2 (4)	8·2 ±0·1 (4)

* Calculated from dose-ratio obtained with vecuronium (100 μM).

Discussion

Vecuronium while having only low activity at muscarinic receptors in atria in functional studies was a ca 10-fold more potent inhibitor in binding studies. Independent confirmation of the potency of vecuronium at cardiac muscarinic receptors is provided by the functional studies in guinea-pig atria of Marshall et al (1980) (pA_2, 4·6 (ACh), 4·9 (pilocarpine)) and by binding studies in rat atria (Dunlap & Brown 1983) (K_I 2 μM). In ileum the difference in affinity constants determined in functional and binding studies was more pronounced (ca 50-fold) so that in the latter experiments no difference in K_I was observable for the two tissues. These findings suggest that while vecuronium can bind to muscarinic receptors with high potency ($K_I \sim 1$ μM) (Table 1) as detected by a ^{3}H antagonist, it does not produce functional inhibition and its action is not likely to occur as a result of competitive inhibition. In atria the slope of the A-S plot was significantly less than 1·0 suggesting that an allosteric mechanism may be involved as with gallamine (Clark & Mitchelson 1976; Stockton et al 1983). In ileum the low potency of vecuronium in functional studies coupled with its low solubility precluded finding evidence for or against such a mechanism.

The findings with himbacine were unexpected as it was reported to behave as a competitive antagonist in functional studies (Gilani & Cobbin 1985) and this was confirmed herein. Further work is necessary to establish the basis for the lack of cardioselectivity in binding studies. As suggested for neuromuscular blocking drugs lack of cardioselectivity could be due to the use of ^{3}H antagonists in binding studies rather than agonists as in functional studies or to the use of homogenates rather than whole cells. Whatever the reason it adds to the list of functional cardioselective muscarinic receptor antagonists that fail to display selectivity in binding studies with [^{3}H](−)-QNB.

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Twenty-one hormones fail to inhibit the brain to blood transport system for Tyr-MIF-1 and the enkephalins in mice

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Abstract—Tyr-MIF-1 (Tyr-Pro-Leu-Gly-amide) and methionine enkephalin are transported intact across the blood-brain barrier by a saturable, stereospecific system. This system has been found to be modulated by a few non-peptide substances and by certain conditions such as ageing and some stresses. We investigated the possibility that hormones structurally unrelated to Tyr-MIF-1 and the enkephalins might also be capable of modulating this transport. Twenty-one hormones were tested including steroids, proteins, glycoproteins, peptides, and thyroid hormones, in doses ranging from 0·01 pmol to 1 nmol/mouse by injecting each hormone directly into the lateral ventricle simultaneously with [^{125}I]Tyr-MIF-1. No clear effect on transport could be established for any of the substances at the doses tested. None of these substances seemed able to act as competitive inhibitors, to share their respective transport systems with Tyr-MIF-1, or to modulate immediately the saturable transport system.

Peptides, like other classes of hormones, can affect the central nervous system (CNS) in several ways including interaction with the blood-brain barrier (BBB). Peptides can cross the BBB (Kastin et al 1976; Banks & Kastin 1985a), alter the BBB transport of non-peptides (Tagliamonte et al 1976; Rudman & Kutner 1978; Goldman & Murphy 1981; Sankar et al 1981; Ermisch et al 1985), or have their BBB transport altered by non-peptides (Banks & Kastin 1986).

Some peptides cross the BBB by diffusing directly across the endothelial/ependymal membranes (Banks & Kastin 1985a, b). Other peptides are transported by saturable, carrier-mediated systems (Banks & Kastin 1984; Michals et al 1986). The best described of these saturable systems for peptides is the one that transports (Banks et al 1986a) Tyr-MIF-1 (Tyr-Pro-Leu-Gly-amide), an antiopiate (Kastin et al 1984, 1985; Galina & Kastin 1986), and methionine enkephalin (Tyr-Gly-Gly-Phe-Met), an opiate.

Although this system has very strict requirements as a transporter, it is regulated in an uncompetitive fashion by non-peptide substances (Banks & Kastin 1986) and altered by ageing (Banks & Kastin 1985c) and some stresses (Banks et al 1988). Thus, endogenous substances may be able to modify the transport system. Such possible modifiers could include hormones, substances known to regulate other systems and affect the CNS. We, therefore, examined many of the major ovarian, adrenal, testicular, hypothalamic, pituitary, and thyroid hormones for their ability to modify this transport system.

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Materials and methods

Transport was determined by the method previously described (Banks et al 1986). Briefly, male ICR mice (Blue Spruce Farms, Altamont NY), 17–20 g, were anaesthetized with ethyl carbamate (urethane). The skull was exposed and a hole 3·0–3·5 mm deep and 1·0 mm lateral and 1·0 mm posterior to the bregma was made into the left lateral ventricle with a guarded 26 gauge needle using a modified technique of Noble et al (1967). One μL of lactated Ringer's solution containing 25000 counts min^{-1} (5·6 fmol) of [^{125}I]Tyr-MIF-1 with or without candidate inhibitors was injected into the ventricle with a guarded Hamilton syringe (Hamilton Co, Reno, NV). Mice were decapitated 10 min after injection and the whole brain except for the pineal and pituitary counted in a gamma counter (Micromedic 4/200, Horsham, PA) for 3 min.

The results were expressed as a percent of the transport (%T) occurring in mice that received no candidate inhibitor, so that substances with no effect have a value of 100, substances with an inhibitory effect on transport have a value of less than 100, and substances with a stimulatory effect have values greater than 100. The equation used to derive %T was:

$$\%T = 100(A - Ex)/(A - Con)$$

where A is the amount of iodinated peptide available for transport, Ex is the amount of radioactivity remaining in the individual brains of mice that received candidate inhibitors, and Con is the mean amount of radioactivity remaining in the brains that received no candidate inhibitors.

The steroids oestrone, oestradiol, oestriol, progesterone, 17-hydroxyprogesterone, testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA) sulphate, and corticosterone were purchased from Sigma (St. Louis, MO) and injected in a lactated Ringer's solution containing 10% ethanol. Mice used as controls for this group also received 10% ethanol in their injectates. Thyroxine and triiodothyronine were also purchased from Sigma. Luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH), prolactin, thyroid stimulating hormone (TSH) and adrenocorticotrophin (ACTH) were a kind gift of the National Hormone and Pituitary Program of the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDKD). Luteinizing hormone releasing hormone (LHRH), thyrotrophin releasing hormone (TRH), angiotensin I, and angiotensin II were purchased from Bachem (Torrance, CA). LH, FSH, TSH, ACTH, LHRH, TRH, angiotensin I, and angiotensin II were dissolved in lactated Ringer solution. GH,