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LETTER TO THE EDITOR

Do adrenergic fibres have muscarinic inhibitory receptors?—a reply

In a recent Letter to the Editor of the Journal of Pharmacy and Pharmacology, Professor J. H. Burn posed the question 'Do adrenergic fibres have muscarinic inhibitory receptors?' (Burn, 1974). Since the article includes several questionable assertions and omits a number of more recent experimental observations very relevant to the argument, we felt obliged to request space in your columns for our comments.

First there is the question of terminology. Burn's claim that, "The terms muscarinic and nicotinic . . . . . do not make any sharp distinction between different types of receptors" is based on a definition of muscarinic and nicotinic properties by the site in the body at which a drug acts, or the end-organ response evoked. However, receptors can only acceptably be defined by their susceptibility to reversible blockade by low concentrations of selective antagonists, and secondarily, by potency ratios of a variety of selective agonists. Thus, the fact that muscarine, pilocarpine and methacholine stimulate both the adrenal medulla and sympathetic ganglia does not indicate nicotinic activity, but rather adrenal medullary (or ganglion) stimulating activity. Further, because these responses are selectively antagonized by low concentrations of atropine, they are correctly referred to as muscarinic. Similarly, the ganglion stimulating effects of nicotine, tetramethylammonium ion or dimethylphenylpiperazinium (DMPP) can justifiably be labelled nicotinic since they are unaffected by atropine, yet abolished by low concentrations of established nicotinic receptor blocking agents (see reviews by Volle, 1966; Trendelenburg, 1967; Haefely, 1972).

A blurred distinction between the two recognized, autonomic cholinergic receptor sites is an essential part of Burn's alternative explanation for the greater release of noradrenaline by acetylcholine from the perfused heart in the presence of atropine (Lindmar, Löffelholz & Muscholl, 1968), and indirectly is an attempt to preserve the cholinergic link hypothesis (Burn & Rand, 1959).

His explanation, which refers to data obtained with the perfused rat mesenteric artery, seems to embody two main points. First, enhancement or inhibition of sympathetic nerve stimulation by acetylcholine results from molecules of the drug initially stimulating the receptors and then, by persisting at the receptor sites, causing blockade. These receptors are the same as those activated by endogenous acetylcholine released as a primary event during sympathetic stimulation (Burn, 1971). Secondly, and here Burn carefully avoids committing himself, the receptors for both acetylcholine and DMPP (which also causes enhancement and inhibition of sympathetic nerve stimulation) are the same, and, one presumes, neither muscarinic nor nicotinic.

However, the evidence available from the experiments on perfused mesentery quoted by Burn do not in fact support his conclusions. Firstly, if only one receptor was involved then the low concentrations of atropine which block the inhibitory responses to acetylcholine (Malik & Ling, 1969a) should also block the enhancing effect. There is no evidence for this on the perfused mesenteric artery, and a similar enhancement of nerve stimulation (and incidentally, noradrenaline) in the rabbit ear artery proved resistant to blockade by atropine (Rand & Varma, 1970). Further evidence for the existence of more than one receptor comes from the observations of Malik & Ling that, although inhibition of nerve stimulation by acetylcholine was

blocked by atropine, the equivalent effect with DMPP was unaffected (Malik & Ling, 1969a, b). Finally, the fact that atropine blocks the inhibitory response to acetylcholine without itself inhibiting nerve stimulation is incompatible with the single receptor hypothesis, since atropine could not prevent exogenous acetylcholine from desensitising the receptors without also inhibiting the activation of these receptors by the hypothetical endogenous acetylcholine.

If one can escape from the confines of the mesenteric artery bed (which seems to be the exception rather than the rule in that the experiments were done at room rather than body temperature), where there was no measurement of actual transmitter release, then there are several papers in the recent literature which have an important bearing on the question posed by Burn. Most of these experiments have been carried out on tissues which satisfy Burn's request that, ". . . to decide whether there are inhibitory receptors, experiments should surely be done on an organ where there is a sympathetic innervation only".

Thus, on the perfused ear artery of the rabbit, a variety of muscarinic agonists have been shown selectively to inhibit the vasoconstriction evoked by peri-arterial nerve stimulation, which was abolished by low concentrations of atropine (Rand & Varma, 1970; Hume, De la Lande & Waterson, 1972). On the same tissue, Steinsland, Furchgott & Kirpekar (1973) combined end-organ response measurements with estimations of the released transmitter. Their results confirmed the earlier observations, and also demonstrated a clear relation between changes in the response to nerve stimulation and endogenous transmitter release. By comparisons of relative potencies of muscarinic agonists and estimation of the dissociation constant of the inhibitory receptor-atropine complex, they were able to conclude that muscarinic agonists act, ". . . on muscarinic receptors . . . (to) interfere with the process by which nerve stimulation causes release of norepinephrine and thus inhibit nerve-evoked vasoconstriction".

At the other end of the vascular spectrum, Vanhoutte, Lorenz & Tyce (1973) used dog isolated saphenous veins in experiments designed to determine if the inhibition of nervous stimulation by acetylcholine resulted from inhibition of noradrenaline release. By direct measurement of tension development and transmitter efflux they were able to conclude, "in the saphenous vein of the dog, the main action of acetylcholine on adrenergic neurotransmission is to decrease the amount of transmitter liberated per stimulus".

Similarly, on the cat perfused spleen, proposed by Burn as a suitable organ for future experiments of this type, evidence has already been obtained which strongly supports the existence of muscarinic inhibitory receptors on adrenergic fibres. Thus, Kirpekar, Prat & others (1972) found that carbachol inhibited the venous outflow of endogenous noradrenaline evoked by sympathetic nerve stimulation, and that the effect was again abolished by atropine.

Finally, recent studies with the rabbit heart have provided further evidence which strengthens the case for the existence of inhibitory muscarinic receptors on adrenergic fibres. An attempt was made to characterize further the receptors of the terminal adrenergic fibres by comparing the potencies of nine compounds with different muscarinic affinities as inhibitors of noradrenaline release after nerve stimulation with their potencies to decrease atrial tension development and ventricular rate (Fozard & Muscholl, 1972). There was good agreement between all nine compounds with respect to their relative potencies on each parameter tested. Since all effects were abolished by low concentrations of atropine, this is unequivocal evidence that the receptors mediating inhibition of atrial tension development, ventricular rate and release of noradrenaline by sympathetic nerve stimulation are muscarinic, and closely similar, if not identical. The nine compounds included 4-(*m*-chlorophenyl-

carbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) and *N*-benzyl-3-pyrrolidyl acetate methobromide (AHR 602), which are selective stimulants of the muscarinic receptors of the superior cervical ganglion which mediate depolarization (Volle, 1966; Trendelenburg, 1967; Haefely, 1972). The results obtained indicated that the muscarinic receptors of the terminal fibres correspond to the receptors of the cell bodies in the ganglion causing hyperpolarization, rather than to those mediating depolarization.

From the information presented above, there are certainly sound experimental data available to invalidate the claim in Burn's final sentence that, "where sympathetic and parasympathetic fibres are not intermingled there seems to be no evidence of 'muscarinic' inhibitory receptors". The important question in this context would seem to be not whether adrenergic fibres have muscarinic inhibitory receptors, but what, if any, is their physiological significance.

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