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LETTERS TO THE EDITOR

Do adrenergic fibres have muscarinic inhibitory receptors?

Lindmar, Löffelholz & Muscholl in 1968 put forward the view that adrenergic fibres have receptors with which muscarinic substances combine to inhibit the release of noradrenaline. The original observation which suggested the idea was that of Hoffmann, Hoffmann & others (1945) who found that when a rabbit isolated heart was perfused with fluid containing atropine, then acetylcholine injected into the aortic cannula caused an increase in the rate and force of the heart beat, and liberation of an adrenaline-like substance (later shown to be noradrenaline) in the outflow. Muscholl therefore conceived that the adrenergic fibres might possess receptors which were stimulated by acetylcholine to inhibit the release of noradrenaline and only when these receptors were blocked by atropine was acetylcholine able to release noradrenaline. Since atropine blocked only muscarinic but not nicotinic receptors, he supposed that the inhibitory receptors were muscarinic.

The proposed inhibitory receptors. The use of the terms "muscarinic" and "nicotinic" to distinguish between receptors raises difficulties. Thus Muscholl considers pilocarpine to be a muscarinic substance, but Dale & Laidlaw (1912) showed that pilocarpine, like nicotine, releases adrenaline from the adrenal gland. Moreover, when a 2% solution of pilocarpine nitrate was applied to the surface of the superior cervical ganglion, it caused a brief dilatation of the pupil, and a prolonged contraction of the nictitating membrane, again acting like nicotine. Then came the work of Ambache, Perry & Robertson (1956) which showed that the original conception of a "muscarinic receptor" required modification in view of the finding that muscarine itself had a nicotine action and could stimulate the perfused superior cervical ganglion when injected into the ganglion. This stimulation was effective in doses which in some experiments were as low as 0·1 μg. Muscarine therefore has "nicotinic" as well as "muscarine" properties, though its action on the ganglion is reversibly blocked by atropine. Now acetylcholine also stimulates the perfused ganglion but its action is not blocked by atropine. We have then acetylcholine, pilocarpine and muscarine which have both muscarinic and nicotinic properties. It seems likely that if metha-

choline were examined for nicotinic properties it would also be found to have them, since it is so closely related to acetylcholine. The terms muscarinic and nicotinic no longer have any precise significance. They do not make any sharp distinction between different kinds of receptors.

There is a different explanation for the greater release of noradrenaline by acetylcholine from the perfused heart in the presence of atropine. In the absence of atropine a large proportion of the acetylcholine molecules stimulate the parasympathetic endings and only a small proportion is available to stimulate the adrenergic endings. When atropine is present all the acetylcholine molecules act on the adrenergic endings.

To gain further evidence to decide whether there are inhibitory receptors, experiments should surely be done on an organ where there is a sympathetic innervation only. Such an organ might be the spleen of the cat or the mesenteric arteries of the rat. The spleen can be perfused and the noradrenaline output measured. The constrictor response to sympathetic stimulation in the mesenteric arteries is extremely regular and indicates the amount of noradrenaline released. As Malik & Ling (1969a) have shown, the infusion of acetylcholine in as small an amount as 50 pg ml^{-1} caused a steadily rising response for 36 min at which point the infusion was stopped. Moreover the method shows the onset of block very clearly. Fig. 2 of their paper shows the large increase in the response to stimulation caused by the infusion of acetylcholine in the concentration of 2 ng ml^{-1} for 15 s, and their Fig. 3 shows the block caused by 5 ng ml^{-1} for 16 min.

The relation between increased response and blocked response is readily understood in terms of the rate theory of drug action of Paton (1961). According to this, for a drug to be an effective stimulant, it must be able to dissociate itself from the receptors as quickly as possible after making contact with them. In this way the number of free receptors is kept high, and the rate at which new molecules of the drug can make contact with them is undiminished. In practice, however, the dissociation of the drug is never as fast as the process of association. Thus while the first application produces stimulation, later applications produce less and less stimulation because molecules of the drug remain attached to the receptors.

The trace in Fig. 1 is taken from an experiment carried out in Ottawa by Dr. K. U. Malik in the presence of Prof. U. S. v. Euler. There is first a series of constrictor responses to stimulation of the sympathetic fibres supplying the perfused mesenteric arteries of the rat. Then acetylcholine (5 ng ml^{-1}) was infused into the perfusion fluid for 4 min with the result that there was almost complete block of the response. However, the four responses to stimulation after the infusion of acetylcholine was stopped, grew progressively bigger until a response more than twice the size of the controls was produced. The rate theory suggests that the normal stimulation at first did not liberate sufficient acetylcholine to occupy all the receptors; but when the infusion of acetylcholine began, almost all receptors were occupied, therefore the next stimulation was ineffective and there was block. The acetylcholine infusion being stopped, receptors gradually became free again, and were acted on by the combined effect of the acetylcholine released by stimulation and the infused acetylcholine which still remained. The effect was optimal after 16 min. The response was then from 2 to 3 times as great as the control responses. Further infusions of acetylcholine repeated this sequence of block followed by supernormal response. These experiments show that the release of noradrenaline by sympathetic stimulation can be steadily augmented by very small amounts of acetylcholine of the order of 50 pg ml^{-1}, or by larger amounts (2 ng ml^{-1}) administered briefly for 15 s. Also the release can be blocked by similar amounts (5 ng ml^{-1}) administered for longer periods such as 4 min or more. There is no suggestion that there are two kinds of receptors, one to

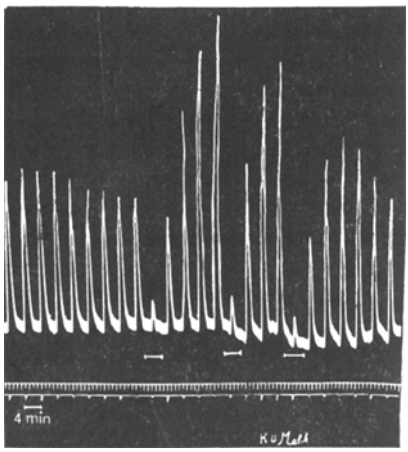

FIG. 1. Trace, by kind permission of Dr. K. U. Malik, of responses of perfused rat mesenteric arteries to stimulation of the sympathetic fibres before and after the infusion of acetylcholine.

accomplish augmentation, and the other to cause block. Both are obtained with the same receptor. Normal responses can be augmented or blocked. Withdrawal of acetylcholine allows the block to pass over to augmented responses. Recently, Dr. K. U. Malik has carried out experiments with methacholine similar to that in Fig. 1. Methacholine infused briefly caused block of the effect of sympathetic stimulation followed by supernormal responses after the infusion stopped.

There is a further matter. If we are attempting to arrive at an understanding of the way in which physiological processes work, in particular the processes which follow stimulation of a nerve, then we cannot substitute for stimulation of the nerve an infusion of acetylcholine. In their experiments, Lindmar & others (1968) tested the effect of acetylcholine, of methacholine and of pilocarpine on the release of noradrenaline by dimethyl phenylpiperazinium (DMPP). DMPP affects the constrictor responses to sympathetic stimulation of the perfused mesenteric arteries in the same way as acetylcholine, except that DMPP is much weaker (Malik & Ling, 1969b). Concentrations having a similar action are acetylcholine 2 ng ml^{-1} and DMPP 300 ng ml^{-1}. Both substances can augment or block in these concentrations according to the length of time for which they are applied.

Starting with a concentration of acetylcholine which did not affect the release of noradrenaline from the heart by DMPP, Muscholl and his colleagues then increased the concentration and found that when they used acetylcholine 10^{-6} g ml^{-1} (i.e. 1000 ng ml^{-1}), the release of noradrenaline by DMPP was very greatly reduced. They concluded that this reduction was due to the action of acetylcholine on "muscarinic inhibitory receptors". But it can be more simply explained by saying that the combination of acetylcholine and DMPP blocked noradrenaline release.

When two drugs having common, but also different, properties are present together, it is rarely, if ever, possible to draw conclusions concerning their joint effect. Certainly acetylcholine, pilocarpine and DMPP all have properties like those of nicotine. Of these substances, only acetylcholine occurs in the body; what is needed is to discover its various relations to noradrenaline. It is known from the experiments with anticholinesterases that acetylcholine releases noradrenaline at the adrenergic terminal as in the adrenal medulla. This was shown for the heart by Huković (1966). It is also known from the work of Paton & Vizi (1969) that noradrenaline reduces the output of acetylcholine. They used the guinea-pig ileum long strip which was stimulated transmurally, and found that the reduction could be as great as 80%. A reduc-

tion was detectable when the concentration of noradrenaline was as low as 2×10^{-7} ml^{-1}.

In cardiac tissue Ehinger, Falck & Sporrong (1970) found adrenergic terminals in close apposition to cholinergic terminals. Thus in rat atrium, taken from an animal treated with 5-hydroxydopamine 4 h previously, they found profiles in contact, one with vesicles with dense cores which were adrenergic, and the other with agranular vesicles which were cholinergic. Presumably the noradrenaline was released from the adrenergic profile by acetylcholine in a layer surrounding the profile as Eränkö, Rechardt & others (1970) found in their pineal results, and not by the acetylcholine released from the cholinergic profile. This might release acetylcholine to act as an inhibitory mechanism of the kind suggested by Muscholl. But where sympathetic and parasympathetic fibres are not intermingled, there seems to be no evidence of "muscarinic" inhibitory receptors.

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The calculation of the tensile strength of tablets

Recently, Rowe, Elworthy & Ganderton (1973), in a calculation of the tensile strength of compacts from the diametral compression test, suggested that an allowance is necessary to enable a comparison of compacts of different porosities to be made. However, the validity of the allowance they propose would seem open to question.

The maximum tensile stress in a cylinder of elastic material loaded in compression along a diameter is given by the equation (Frocht, 1948) where P is the applied

$$\sigma_t = \frac{2P}{\pi Dt}$$

diametral load, D is the diameter of the cylinder and t is its thickness. This maximum stress is approximately constant over almost the entire length of the loaded diameter.

At failure the value of the maximum tensile stress σ_t represents the tensile strength of the sample. For non-ideal materials under non-ideal test conditions, such as the testing of pharmaceutical tablets, the stress conditions will also not be ideal. Nevertheless, as shown by Peltier (1954), for various assumed stress distributions, the tensile stress can be held uniform over a reasonable proportion of the diameter if the width of the loading area is less than 1/5 the specimen diameter. Rudnick, Hunter & Holden (1963) have pointed out that, because of the departure from ideal behaviour,