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Effect of muscarinic agonists on the release of [^{3}H]noradrenaline from the guinea-pig perfused heart

The administration of low doses of acetylcholine or muscarinic agonists decreases the release of noradrenaline following sympathetic nerve stimulation to the various tissues (Löffelholz & Muscholl, 1969; Malik & Ling, 1969; Steinsland, Furchgott & Kirpekar, 1973; Vanhoutte, Lorenz & Tyce, 1973). This has given rise to the hypothesis that acetylcholine released from a cholinergic nerve inhibits the release of noradrenaline from an adjacent adrenergic nerve via an inhibitory action on muscarinic receptors. We now report that both acetylcholine and methacholine prevent the release of [^{3}H]noradrenaline (^{3}H-NA) by nicotine in the guinea-pig perfused heart and show that acetylcholine fails to release [^{3}H]noradrenaline from the guinea-pig perfused heart except when used in very high concentrations (10^{-3}M). However, if atropine (10^{-5}M) is added to the perfusion solution, acetylcholine at 10^{-6}M will readily release ^{3}H-NA.

Hearts from male guinea-pigs (200–400 g) were removed under sodium pentobarbitone anaesthesia and immediately connected to a coronary perfusion apparatus (Anderson-Craver; Metro Scientific Co.) via the aorta. The normal perfusion medium contained (mmol litre^{-1}): NaCl, 119·8; KCl, 5·63; $CaCl_2$, 2·16; $MgCl_2$, 2·10; dextrose, 100 and $NaHCO_3$, 25·0. The solution was bubbled with 5% CO_2 in oxygen; the temperature was maintained at 38 $\pm$ 1° and pH 7·32–7·45. Hearts were perfused at 6·0 $\pm$ 1·0 ml min^{-1} with a Harvard perfusion pump.

After an equilibration period, hearts were perfused with 1·0 ng ml^{-1} of (−)-[^{3}H]-noradrenaline (specific activity 5·8 Ci mmol^{-1}) for 20 min to label the endogenous store. The perfusate was changed to a noradrenaline-free medium and the efflux of ^{3}H-NA was collected and analysed.

Drugs used were: (−)-[7-^{3}H]noradrenaline (New England Nuclear); acetylcholine chloride, acetyl-β-methylcholine chloride (methacholine) (Sigma); nicotine hydrochloride (K & K Labs, Inc., Plainview, N.Y.) and atropine sulphate (Mallinckrodt).

Two types of experiments were carried out. First, the effect of the addition of acetylcholine or methacholine to the perfusion medium on the release of ^{3}H-NA by

Table 1. *The effect of nicotine, acetylcholine or methacholine alone as well as the effect of nicotine in the presence of acetylcholine and methacholine on the efflux of ^{3}H-NA from the guinea-pig perfused heart.*

Collection period[a]	^{3}H–NA d min^{-1} × 10^{-3}					
	Control	Nic[b]	Ach[b]	Meth[b]	Ach[c] + Nic	Meth[c] + Nic
2	80	79	85	82	78	83
4	30	35	34	32	29	40
6	12	11	15	13	14	15
8	9	10	12	11	10	12
10	6	7	9	9	9·5	11
12	5·5	6	8	8·5	8·9	8·9
14	5·4	5·9	7·5	6·9	7·4	8·2
16	4·9	5·4	6·5	6·3	6·4	8·0
18	4·8	4·9	6·3	5·9	6·2	7·9
20[b]	4·2	4·9	6·0	5·8	6·0	7·5
22	4·1	99·0	5·9	5·6	14·5	14·2
24	3·9	50·1	5·8	4·9	5·8	6·0
26	3·8	4·2	5·0	4·3	5·5	6·0
30	3·7	4·0	4·9	4·2	5·2	5·9

[a] Time in min after switching from medium containing ^{3}H–NA to normal medium.
[b] Nicotine (0·86 × 10^{-4}M), acetylcholine (10^{-5}M), or methacholine (10^{-5}M) infused for 1 min.
t Acetylcholine or methacholine were added 10 min before injection of nicotine. Concentrations were the same as in b.

nicotine was examined. One or other of the choline esters was added in a concentration of 10^{-5}M to the perfusion medium 10 min before a 1 min infusion of nicotine at a total concentration of 0·86 × 10^{-4}M (we had found that nicotine caused a dose-related release of ^{3}H-NA; we had also found that it released ^{3}H-NA from adrenergic nerves, Westfall & Brasted, 1972). The perfusate effluents from the hearts were continuously collected and analysed for ^{3}H-NA by liquid scintillation spectrometry after the purification of ^{3}H-NA by alumina column chromatography.

The second type of experiment determined the effect of acetylcholine in releasing ^{3}H-NA in the absence or presence of atropine. Acetylcholine was infused in various concentrations from 10^{-7} to 10^{-3}M over 1 min. These experiments were then repeated in hearts receiving 10^{-5}M atropine in the perfusion medium. Perfusate effluents were collected and analysed as described above.

Table 1 shows the effect of nicotine, methacholine or acetylcholine injected alone as well as the effect of nicotine administered in the presence of acetylcholine or methacholine on the release of ^{3}H-NA from the guinea-pig perfused heart. Nicotine itself produces a marked release of ^{3}H-NA but neither acetylcholine nor methacholine had any effect. If the same concentration of acetylcholine or methacholine was added to the perfusion medium before the addition of nicotine, the releasing effect was greatly attenuated.

Fig. 1 shows the effect of various concentrations of acetylcholine on the release of ^{3}H-NA alone or in the presence of atropine. Acetylcholine had no releasing effect until a concentration of 10^{-3}M was added. However, in the presence of 10^{-5}M atropine, it caused a dose-related release of ^{3}H-NA at a concentration of 10^{-6}M.

Acetylcholine or other muscarinic agonists decrease the release of noradrenaline following nerve stimulation. This has been shown in perfusion experiments using the rabbit heart (Löffelholz & Muscholl, 1969), the ear artery (Malik & Ling, 1969; Steinsland & others, 1973), the cat spleen (Kirpekar, Prat & others, 1972), the dog saphenous vein (Vanhoutte & others, 1973) and superfused brain slices (Westfall, 1974). This has given rise to the hypothesis that acetylcholine released from a

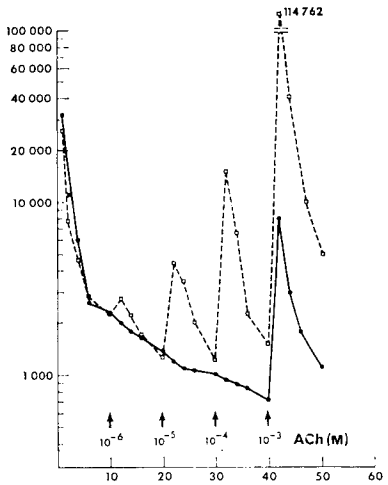

FIG. 1. The efflux of ^{3}H–NA from the perfused guinea-pig heart is plotted on the ordinate (d min^{-1}) against time in min on the abscissa. At each arrow acetylcholine was infused for 1 min at the final concentration mentioned in control hearts (●——●) or hearts in which 10^{-5}M atropine had been added to the perfusion medium (□- - -□).

cholinergic nerve inhibits the release of noradrenaline from an adjacent adrenergic nerve via an action on muscarinic receptors. This can be viewed as a contralateral control mechanism located at the level of the nerve terminal whereby the neurotransmitter from one neuron regulates the release of another neurotransmitter and therefore exerts a modulating influence. Our results are consistent with this hypothesis and demonstrate that muscarinic agonists also decrease the release of noradrenaline induced by nicotine in the guinea-pig isolated heart. Further, evidence is presented suggestive that the muscarinic inhibitory receptors are much more sensitive to acetylcholine than are the nicotinic receptors. Only when a high concentration of acetylcholine is administered is there a release of noradrenaline. However, by blocking the muscarinic receptors with atropine, acetylcholine at a low concentration then produces a release of noradrenaline. This report adds supportive evidence for the muscarinic inhibitory mechanism as a means of regulating the release of noradrenaline.

This work was supported by grants from U.S. Public Health Service NS 10260–01 and the Council for Tobacco Research, U.S.A.

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January 7, 1974

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