*Short communications*

# Comparison of effects of six cholinomimetic drugs on inhibition of uptake of $^3$H-(±)-noradrenaline by guinea-pig atria

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The effects of *N,N*-dimethyl-*N'*-phenylpiperazinium (DMPP), 4-(*m*-chlorophenylcarbamoyloxy) - 2 - butynyltrimethylammonium chloride (McN-A-343), pilocarpine, acetylcholine, methacholine and nicotine in inhibiting the uptake of $^3$H-(±)-noradrenaline by guinea-pig atria were compared. In concentrations of $1 \times 10^{-4}$M, the percentage inhibitions were as follows: DMPP, 89·1%; McN-A-343, 78·7%; pilocarpine 43·5%; acetylcholine, 35·7%; methacholine, 32·9%; nicotine, 21·6%.

**Introduction.** — Two cholinomimetic drugs, DMPP (*N,N*-dimethyl-*N'*-phenylpiperazinium) and McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy) - 2 - butynyl - trimethylammonium) are potent inhibitors of the uptake of noradrenaline by guinea-pig atria (Allen, Rand & Story, 1972). In isolated artery preparations, these drugs can cause increases or decreases in responses to sympathetic nerve stimulation, the particular effect depending on the frequency of stimulation and the concentration of drug (Malik & Ling, 1969a; Rand & Varma, 1971). Similarly acetylcholine, depending on the concentration and period of contact, may either reduce or enhance the responses of isolated artery preparations to sympathetic nerve stimulation (Malik & Ling, 1969b; Rand & Varma, 1970). The facilitatory action of acetylcholine has been attributed to increased noradrenaline release and held to be in accord with the hypothesis of Burn & Rand (1959), that acetylcholine mediates the release of noradrenaline by nerve impulses. However, if inhibition of noradrenaline uptake (and re-uptake) were a general property of cholinomimetic drugs, it could explain the facilitatory effects of acetylcholine and related drugs on the responses to sympathetic nerve stimulation in isolated artery preparations. It was important to establish, therefore, the effects of acetylcholine on noradrenaline uptake. The results showed that acetylcholine and three other cholinomimetics were considerably less active than DMPP and McN-A-343 in inhibiting noradrenaline uptake in guinea-pig atria.

**Methods.**—The uptake of $^3$H-(±)-noradrenaline by guinea-pig atria and the effects of drugs on the uptake were measured as described by Allen *et al.* (1972). Atria were incubated with $^3$H-(±)-noradrenaline ($1{\cdot}3 \times 10^{-7}$M, 0·5 μCi/ml) in Krebs–Henseleit solution at 37° C for 20 minutes. The atria were then washed for 10 min in Krebs–Henseleit solution and the accumulation of total tissue radioactivity determined by liquid scintillation counting. The effects of drugs on the uptake of $^3$H-noradrenaline were investigated by preincubating atria with the drugs for 15 min prior to the addition of the labelled noradrenaline.

The additional drugs used were acetylcholine perchlorate (B.D.H.), methacholine chloride (Sigma), nicotine hydrogen (+)-tartrate (B.D.H.), and pilocarpine nitrate (Macfarlan Smith). Tritiated racemic noradrenaline, 7-($^3$H)-(±)noradrenaline hydrochloride (specific activity, 3·8 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

TABLE 1. *Effects of* $1 \times 10^{-4}$M *concentrations of cholinomimetic drugs on the uptake of* $^3$*H-(±)-noradrenaline by guinea-pig atria*

| Drug | Percentage inhibition of uptake ± S.E. (number of experiments)* |
|---|---|
| DMPP† | 89·1 ± 1·1 (5) |
| McN-A-343† | 78·7 ± 2·5 (4) |
| Pilocarpine | 43·5 ± 8·6 (5) |
| Acetylcholine | 35·7 ± 5·0 (4) |
| Methacholine | 32·9 ± 4·5 (4) |
| Nicotine | 21·6 ± 5·0 (3) |

* Control uptake = 2289 ± 113 d/min per mg of tissue. † These data are from different sets of experiments from those reported by Allen, Rand & Story (1972).

**Results.**—Table 1 summarizes the effects of DMPP, McN-A-343, pilocarpine, acetylcholine, methacholine and nicotine, each in a concentration of $1\times10^{-4}$M on the uptake of $^3$H-(±)-noradrenaline by guinea-pig atria. In each case there was a statistically significant inhibition of uptake, although the effect was small except with DMPP and McN-A-343.

**Discussion.** — The literature contains only passing reference to direct experiments on the effects of cholinomimetic drugs on noradrenaline uptake. Lindmar, Löffelholz & Muscholl (1968) reported that acetylcholine, alone or in the presence of atropine, does not affect the removal of noradrenaline from the fluid perfusing isolated rabbit hearts. Nicotine is reported to be without effect on the uptake of $^3$H-noradrenaline (Nedergaard & Bevan, 1969; Westfall, 1971). Hamilton (1971) suggested that muscarine, oxotremorine, arecoline and muscarine promote the uptake of isoprenaline, which then releases noradrenaline.

If the findings on inhibition of noradrenaline uptake by cholinomimetics can be extrapolated from atria to arteries, it is unlikely that the facilitation of responses to sympathetic nerve stimulation produced by cholinomimetics in artery preparations is due to inhibition of uptake. The facilitation is produced by very low concentrations of acetylcholine (Malik & Ling, 1969a), and after termination of infusions of acetylcholine, methacholine, muscarine, carbachol and arecoline (Rand & Varma, 1970). During infusions of acetylcholine and methacholine in concentrations of about 0·1 to 5 μg/ml (ca $5\times10^{-7}$ to $2{\cdot}5\times10^{-5}$M), responses of the artery are depressed, the effect probably being due to the muscarinic inhibition of noradrenaline release (Lindmar *et al.*, 1968). However, with larger concentrations of acetylcholine and methacholine they tend to increase towards control levels (Rand & Varma, 1971); this effect may well be due to inhibition of re-uptake of noradrenaline, especially since responses to injected noradrenaline were considerably enhanced by such concentrations. These suggestions must remain speculative pending the results of noradrenaline uptake studies in artery preparations. Preliminary experiments indicate an avid uptake of $^3$H-noradrenaline by rabbit ear artery segments, but there are very considerable differences between preparations even when they are taken from the ears of the same rabbit.

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