Thus propranolol, oxprenolol and practolol reduce the isometric tension induced by electrical stimulation in both isolated skeletal and cardiac muscle. However, the concentrations used to produce these effects are consistent with the concentrations of the drugs known to produce non-specific effects *in vitro*. These concentrations are greater than those used by Harry *et al.* (1971) in the intact dog and suggest that the actions of these three β-adrenoreceptor blocking agents on the effects of isoprenaline on the intact dog heart are not related to the effects reported here on isolated muscle preparations.

REFERENCE

HARRY, J. D., KAPPAGODA, C. T., LINDEN, R. J. & SNOW, H. M. (1971). Effects of β-adrenoceptor blocking drugs on the chronotropic and inotropic actions of isoprenaline on the acutely denervated dog heart. *Br. J. Pharmac.*, **41**, 387*P*.

Effects of several muscarinic agonists on cardiac performance and the release of noradrenaline from the sympathetic nerves of the rabbit heart

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There are on the terminal adrenergic fibres of the rabbit heart muscarinic receptors which, upon excitation, can inhibit the release of noradrenaline evoked both by nicotinic drugs and electrical stimulation of the sympathetic nerves (Muscholl, 1970). The specificity of these receptors has been investigated by comparing the potencies of several compounds with different muscarinic intrinsic activities on systolic atrial tension development, ventricular frequency and neuronal noradrenaline release.

Atrial and ventricular tensions (Fozard & Muscholl, 1971), ventricular frequency and coronary flow were measured in Langendorff rabbit hearts perfused at 60 cm water and 35·5°C. Concentration-effect curves to acetylcholine, oxotremorine, pilocarpine, N-methyl-1,2,5,6, tetrahydro-nicotinic acid propylyl ester (MH-1, Mutschler & Hultzsch, 1971), N-benzyl-3-pyrolidyl acetate methobromide (AHR 602) and 4-(m-chlorophenylcarbamoyloxy)-2-butyryltrimethylammonium chloride (McNeil-A-343) were established by perfusion with increasing concentrations of each compound for 1 min at 10 min intervals. In separate experiments, noradrenaline release into the perfusates was induced by postganglionic sympathetic nerve stimulation -NS- (Hukovic & Muscholl, 1962) (600 rectangular pulses, 1 ms, 10 Hz, supramaximal voltage) and 1,1-dimethyl-4-phenylpiperazinium-DMPP- ($9{\cdot}5 \times 10^{-5}$ M). Muscarinic agents were perfused 1 min before and during the 3 min collection period for NS or DMPP. Noradrenaline was estimated fluorimetrically after absorption on, and elution from, alumina. The results are summarized in Table 1.

TABLE 1. *pD_2 values (Ariëns, 1964) with potencies relative to acetylcholine (100) for inhibition of:*

					Noradrenaline release by			
Compound	Atrial tension	n_1	Ventr. rate	n_1	NS	n_2	DMPP	n_2
MH-1	8·01 (316)	5	6·91 (1098)	5	6·22 (417)	7	5·68 (186)	9
Oxotremorine	7·88 (234)	5	6·78 (813)	5	6·04 (275)	6	5·38 (93)	7
Acetylcholine	7·51 (100)	6	5·87 (100)	6	5·65 (100)	10	5·41 (100)	10
Pilocarpine	5·52 (1·0)	4	4·42 (2·2)	4	3·09 (0·3)	6	3·45 (1·1)	8
McNeil-A-343	4·46 (0·09)	4	<3·31 (<0·28)	4	facilitation†	8	5·25 (69)†	6
AHR 602	<3·30 (<0·006)	4	<3·30 (<0·27)	4	facilitation†	7	4·25 (6·9)†	6

n_1 = number of individual concentration-effect curves. n_2 = number of individual estimations used o calculate regression lines. † = effect not antagonized by atropine ($7{\cdot}2.10^{-7}$ M).

The results demonstrate the general similarity between the muscarinic receptors mediating inhibition of noradrenaline release, atrial tension development and ventricular frequency. In particular, by analogy with sites in the sympathetic ganglion, inhibition of noradrenaline release is more likely to be the result of muscarinic-induced hyperpolarization than depolarization, and McNeil-A-343 and AHR 602 show relative specificity for the receptors (Trendelenburg, 1967).

REFERENCES

ARIËNS, E. J. (Ed.). (1964). *Molecular Pharmacology: The Mode of Action of Biologically Active Compounds.* pp. 153–156. London: Academic Press.

FOZARD, J. R. & MUSCHOLL, E. (1971). The differential recording of atrial and ventricular tension in the perfused rabbit heart. *Naunyn-Schmiedebergs Arch. Exp. Path. Pharmak.*, **270**, 319–325.

HUKOVIC, S. & MUSCHOLL, E. (1962). Die Noradrenalin-Abgabe aus dem isolierten Kaninchenherzen bei sympathetischer Nervenreizung und ihre pharmakologische Beinflussung. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **244**, 81–96.

MUSCHOLL, E. (1970). Cholinomimetic Drugs and the Adrenergic Transmitter. Symposium on: *New Aspects of Storage and Release Mechanisms of Catecholamines*, Ed. Schümann, H. J. & Kroneberg, G. pp. 168–186. Berlin: Springer.

MUTSCHLER, E. & HULTZSCH. (1971). Über Struktur-Wirkungs-Beziehungen von ungesättigten Estern des Arecaidins und Dihydroarecaidins. *Arzneimittelforschung*, in the Press.

TRENDELENBURG, U. (1967). Some aspects of the pharmacology of autonomic ganglion cells. *Ergebn. Physiol.*, **59**, 1–85.

Demethylation of 3-*O*-methyldopa

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It has recently been shown that administration of L-3-*O*-methyl,4-hydroxyphenylalanine (3-*O*-methyldopa; 'OMD'), a major metabolite of L-dihydroxy-phenylalanine (L-dopa), leads to the formation of dopamine in the rat brain (Bartholini, Kuruma & Pletscher, 1971). These authors suggest that the increase is the result of initial demethylation of OMD to L-dopa which is then decarboxylated to dopamine, since OMD is a poor substrate for decarboxylation and does not appear to be directly converted to 3-*O*-methyldopamine in appreciable quantities either *in vitro* (Ferrini & Glasser, 1964) or *in vivo* (Bartholini, Kuruma & Pletscher, 1971).

In *O*-demethylation the methyl group is oxidized, via formaldehyde, to carbon dioxide which is eliminated in expired air. Thus, in order to measure directly the extent and rate of *O*-demethylation we have enzymatically synthesized methyl labelled ^{14}C-OMD from L-dopa, using a partially purified catechol-*O*-methyl transferase (Axelrod & Tomchick, 1958) and S-adenosylmethionine (methyl-^{14}C). One microcurie of ^{14}C-OMD was given intraperitoneally to rats maintained in an airtight chamber ('Metabowl'—Jencons) permitting continuous collection of expired CO_2, urine and faeces. After a delay of about 4 h radioactivity began to be excreted slowly as ^{14}C-CO_2 in the expired air. A total of 15–20% of the dose was recovered in the expired air over 3–4 days, thus directly demonstrating that a significant fraction of the administered OMD is demethylated *in vivo*. During the same period 65% of the dose was excreted in the urine and 8% in faeces. We were unable to demonstrate oxidative demethylation of OMD by liver or brain *in vitro*, even though it is well established that liver preparations can *O*-demethylate several foreign compounds (Gillette, 1966). Since the initial delay and slow rate of excretion of ^{14}C-CO_2 might have been due to demethylation of OMD in the gut after excretion in the bile, experiments were repeated in rats with biliary fistulae. In these animals 20% of the dose was excreted in the bile during the