heart rate and carotid occlusion response after decerebration may indicate the presence of some degree of inhibitory tone to medullary cardiovascular centres by the higher centres. This preparation is well suited for investigating the site of action of drugs that affect the cardiovascular system through a central mechanism.

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Selective block of cardiovascular adenylate cyclase activation *in vivo*

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Stimulation of β-adrenoceptors by sympathomimetic drugs increases tissue cyclic 3′-5′-adenosine monophosphate (cyclic AMP) concentrations by activating an adenylate cyclase system which regulates cyclic AMP biosynthesis (Robison & Sutherland, 1970). The increase in cyclic AMP concentrations is presumed to mediate pharmacological responses to sympathomimetic drugs despite evidence of dissociation between inotropic responses and adenylate cyclase activation in cardiac tissue (Wastila, Su & others, 1972; Benfey, Kunos & Nickerson, 1974).

The present report describes an investigation of the ability of β-adrenoceptor blocking agents to block adenylate cyclase activation by isoprenaline in cardiac and vascular tissues *in vivo*. The blocking agents studied are known to have different selectivities in blocking cardiostimulant and vasodilator responses to isoprenaline *in vivo*.

Female white rats (Fisher Strain), 150–250 g, were anaesthetized by an intraperitoneal (i.p.) injection of pentobarbitone sodium (50 mg kg^{-1}) and anaesthesia maintained by additional doses as needed. The femoral vein was isolated and cannulated and the trachea cannulated to facilitate respiration. The animals were killed by removal of the heart (atria and ventricles) and aorta approximately 50 min after an intraperitoneal injection of physiological saline or theophylline (45 mg kg^{-1}) and 1 min after an intravenous injection of physiological saline or (±)-isoprenaline (10 μg kg^{-1}). Some animals received an injection of propanolol (0·5 mg kg^{-1}), practolol (4 mg kg^{-1}) or H 35/25 (4 mg kg^{-1}) 15 min before the isoprenaline injection.

The doses of isoprenaline and blocking agents used were selected in preliminary experiments using theophylline-pretreated rats anaesthetized with pentobarbitone. Heart rate and blood pressure were recorded in these experiments on a Hewlett Packard Model 7700 polygraph via a Statham PT06 transducer attached to a cannula inserted in a carotid artery. In these experiments, isoprenaline (10 μg kg^{-1}) significantly increased heart rate and decreased diastolic blood pressure; both of these effects were abolished by pretreatment with propranolol (0·5 mg kg^{-1}). Practolol (4 mg kg^{-1}) significantly reduced the effect of isoprenaline on heart rate but not on blood pressure. H 35/25 (4 mg kg^{-1}) significantly reduced the effect of isoprenaline on blood pressure but not on heart rate. In all of the animals in which heart rate and blood pressure were measured, blocking agents were administered in the same time sequence as in the animals from which heart and aorta samples were removed, i.e. 25 min after theophylline (45 mg kg^{-1}, i.p.) and 15 min before isoprenaline (10 μg kg^{-1}, i.v.).

The heart and aorta samples were homogenized with glass tissue homogenizers in cold trichloroacetic acid solution, centrifuged and the resulting supernatants washed with diethyl ether as described by Brown, Albano & others (1971). The cyclic AMP content of heart and aorta extracts was then determined by a protein-binding assay procedure based on the competition between unlabelled (tissue) and tritium-

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labelled cyclic AMP for a specific cyclic AMP binding protein (Gilman, 1970). Unbound cyclic AMP was removed by adsorption on charcoal (Brown & others, 1971) and protein-bound cyclic AMP determined by counting for 5 min in PCS (Amersham/Searle) cocktail with a Beckmann Model LS-100 liquid scintillation counter. Tissue cyclic AMP concentrations were obtained by plotting on a calibration curve constructed from five known cyclic AMP concentrations and the results expressed as pmol of cyclic AMP g^{-1} of tissue (wet weight). A separate calibration curve was prepared for each assay.

The drugs used were pentobarbitone sodium (Abbott), theophylline, (±)-isoprenaline hydrochloride (Sigma), propranolol hydrochloride (Ayerst), practolol (ICI) and H 35/25 (1-(4-methylphenyl)-2-isopropyl aminopropanol) hydrochloride (Axel Kistner AB). The doses of the theophylline, isoprenaline and practolol are in terms of the bases; the doses of the other drugs are in terms of the salts. Reagents used for determination of cyclic AMP were all obtained from Amersham/Searle Corporation.

Statistical analyses were made using the Student's *t*-test for unpaired data. A P value of less than 0·05 was considered as significant.

Table 1 shows the mean cyclic AMP concentration ± standard error for cardiac and vascular tissues obtained from animals injected with saline (saline controls) or theophylline (45 mg kg^{-1}, i.p.) (theophylline controls) 50 min before removal of tissues. As shown in Table 1, theophylline pretreatment significantly increased cyclic AMP concentrations in both cardiac and vascular tissues compared to saline controls.

Table 1. *Cardiovascular cyclic AMP concentrations in rats pretreated with saline (saline controls) or theophylline (45 mg kg^{-1}, i.p.) (theophylline controls) 50 min before removal of tissues.* n = number of animals.

Group	n	Cyclic AMP concn (pmol g^{-1} tissue) Heart	Aorta
Saline controls	4	296 ± 12	933 ± 66
Theophylline controls	6	500 ± 43*	1244 ± 60*

* Significantly different from saline controls.

Table 2 shows the mean cyclic AMP concentrations ± standard error for cardiac and vascular tissues obtained from animals receiving isoprenaline (10 μg kg^{-1}, i.v.) after pretreatment with saline (saline + iso) or with theophylline (45 mg kg^{-1}, i.p.) (theophylline + iso). Cardiovascular cyclic AMP concentrations were significantly higher in the theophylline + iso group than in theophylline controls. Cyclic AMP concentrations in the saline + iso group were not significantly higher than cyclic AMP concentrations in saline controls.

Table 3 shows the mean cyclic AMP concentrations ± standard error for cardiac and vascular tissues obtained from animals pretreated with theophylline (45 mg kg^{-1}, i.p.) and injected with isoprenaline (10 μg kg^{-1}, i.v.) at 15 min after intravenous administration of propranolol, practolol or H 35/25. Propranolol completely blocked the effect of isoprenaline on cyclic AMP concentrations in both cardiac and vascular tissues. Practolol significantly decreased the effect of isoprenaline on cardiac cyclic AMP concentrations but did not alter the increase in vascular cyclic AMP concentrations produced by isoprenaline. In contrast to practolol, H 35/25 blocked the effect of isoprenaline on vascular cyclic AMP concentrations but did not significantly decrease the effect of isoprenaline on cardiac cyclic AMP concentrations.

Pretreatment with theophylline *in vivo* increased cyclic AMP concentrations and enhanced their increase when produced by isoprenaline in both cardiac and vascular tissues. These results are in contrast to its failure to alter cyclic AMP concentrations or to potentiate their increase when produced by noradrenaline in perfused rat heart (McNeill, Coutinho & Verma, 1974). The results suggest that theophylline pretreatment of isoprenaline-treated rats as used in this study prolonged the increase in tissue cyclic AMP concentrations produced by adenylate cyclase activation. This was presumably due to inhibition of phosphodiesterase metabolism during the time interval before tissue isolation and extraction.

Table 2. *Cardiovascular cyclic AMP concentrations in rats injected with isoprenaline (10 μg kg^{-1}, i.v.) at 1 min before removal of tissues.* Animals were pretreated with saline (saline + iso) or theophylline (45 mg kg^{-1}, i.p.) (theophylline + iso) 50 min previously. n = number of animals.

Group	n	Cyclic AMP concn (pmol g^{-1} tissue) Heart	Aorta
Saline + iso	4	380 ± 57	1031 ± 31
Theophylline + iso	6	1910 ± 147*	1713 ± 59*

* Significantly different from controls.

The increase in both cardiac and vascular cyclic AMP concentrations produced by isoprenaline in theophylline-pretreated animals was non-selectively blocked by propranolol which is known to block catecholamine-induced adenylate cyclase activation in both cardiac (Burges & Blackburn, 1972; Murad, 1973) and in vascular (Volicer & Hynie, 1971; Triner, Vulliemoz & others, 1972) tissues *in vitro* and to block cardio-

Table 3. *Cardiovascular cyclic AMP concentrations in rats injected with isoprenaline (10 mg kg^{-1}, i.v.) at 1 min before removal of tissues.* Animals were pretreated with theophylline (45 mg kg^{-1}, i.p.) 50 min previously and with propranolol (0·5 mg kg^{-1}, i.v.) (iso + propranolol), practolol (4 mg kg^{-1}, i.v.) (iso + practolol) or H 35/25 (4 mg kg^{-1}, i.v.) (iso + H 35/25) 15 min previously. n = number of animals.

Group	n	Cyclic AMP concn (pmol g^{-1} tissue) Heart	Aorta
Iso + propranolol	6	490 ± 31*	1186 ± 58*
Iso + practolol	6	751 ± 38*	1716 ± 55
Iso + H 35/25	6	1638 ± 190	1212 ± 81*

* Significantly different from theophylline + iso group.

stimulant and vasodilator responses non-selectively *in vivo*. Practolol, which selectively blocks cardiostimulant responses to isoprenaline *in vivo* (Levy & Wilkenfeld, 1969) is known to block catecholamine-induced adenylate cyclase activation in cardiac tissues *in vitro* (Burges & Blackburn, 1972; Murad, 1973). The ability of practolol to block catecholamine-induced adenylate cyclase activation in vascular tissues *in vitro* does not appear to have been studied. H 35/25 is known to selectively block vasodilator responses to isoprenaline *in vivo* (Levy & Wilkenfeld, 1969) but the ability of this blocking agent to alter catecholamine-induced adenylate cyclase activation *in vitro* does not appear to have been studied in either cardiac or vascular tissues.

The block of isoprenaline-induced increases in cardiac and vascular cyclic AMP concentrations by propranolol suggests that, in the rat heart and aorta, inhibition of adenylate cyclase activation is associated with block of cardiostimulant and vasodilator responses to β-adrenoceptor stimulation *in vivo*. Adenylate cyclase activation and pharmacological responses appear to be parallel but possibly independent events resulting from local β-adrenoceptor stimulation.

In the present study, practolol and H 35/25 produced a selective block of adenylate cyclase activation by isoprenaline in heart and aorta which is consistent with the selective blocking actions of these drugs on cardiostimulant and vasodilator responses to isoprenaline *in vivo*. These results support the concept that β-adrenoceptors in cardiac and vascular tissues are differentiated into distinct subtypes, each subtype mediating local adenylate cyclase activation, and, presumably, pharmacological responses to sympathomimetic drugs. The results do not necessarily confirm the classification of adrenoceptors into β_1 and β_2 subtypes as proposed by Lands, Arnold & others (1967) since other tissues may have β-adrenoceptors which are not identical to those in either heart or aorta (Bristow, Sherrod & Green, 1970).

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