conditions was 77 ± 3% (mean ± s.e. mean, $n = 15$). No pressor activity was detectable in zero time incubation samples or in samples incubated at 4°C.

The pH optimum of the rat brain iso-renin dog substrate system was noted to be in the region of pH 4.4 to 4.8, and the apparent Michaelis constant K_m, of the system about 0.5 μM (expressed as angiotensin II). With the level of substrate used for routine assay, the reaction appeared to be first order and the generation of angiotensin was linear for over 24 hours. Using this technique brain iso-renin activity in normal male Wistar rats has been found to be in the range 90–100 ng Angiotensin II g brain^{-1} hour^{-1}. This level of activity should be sufficient to allow an investigation of regional differences in brain renin activity and of any changes induced by therapeutic agents or by changes in physiological status.

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The role of transmembrane calcium flux in the adrenergic response of the isolated frog heart

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Kunos & Szentivanyi (1968) have reported that the response of the isolated winter frog heart to catecholamines alters with temperature in a manner consistent with a direct, temperature-dependent interconversion of α- and β-adrenoceptors. Buckley & Jordan (1970) postulated the coexistence of both receptor types whose activation was achieved at different temperatures. In support of this postulate, the relative potencies of adrenaline and isoprenaline were reversed when heart temperature was lowered. The aim of the present study was to further characterize the adrenergic response at 23°C and 6°C in the winter frog heart.

Isolated hearts were suspended in frog Ringer's solution at 6°C or 23°C and treated with 1 μM [*N*-(3,4-dimethoxyphenethyl)-*N*-methylamino]-α-(3,4,5-trimethoxyphenyl)α-isopropylvaleronitrile hydrochloride (D600 hydrochloride) for 30 minutes. Rate and contraction amplitude of the heart decreased at both temperatures. The greater rate decreases at 23°C or amplitude reductions at 6°C did not differ sufficiently to indicate temperature-mediated dissociation of these effects. For this reason, decrease in performance was measured as percentage change in work output (amplitude × rate). The reduction in work output obtained with D600 at 23°C was much greater than at 6°C (see Table 1). Subsequent treatment with isoprenaline at 23°C resulted in restoration of control values while adrenaline only partly overcame the reduction. In hearts studied at 6°C, adrenaline was now more effective than isoprenaline.

It has been proposed that D600 inhibits excitation-contraction coupling by selective blockade of calcium transmembrane flux into excited myocardial fibres (Fleckenstein, 1971; Kohlhardt, Bauer, Krause & Fleckenstein, 1972). D600 has previously been used to differentiate between the inotropic actions of isoprenaline and phenylephrine in isolated guinea-pig ventricle (Ledda, Marchetti & Mugelli, 1975). The interactions described above would indicate that at

Table 1 Percentage changes in work output (± s.e. mean) produced by D600 (1×10^{-6} M) and modified with adrenaline (1.2×10^{-5} M) or isoprenaline (1.2×10^{-5} M)

	D600	*ADR*	*ISOP*
23°C	−65.3 ± 4.6 (6)		+9.5 ± 10.00
	−67.8 ± 3.6 (6)	−31.5 ± 13.3	
6°C	−12.5 ± 2.5 (4)		−4.0 ± 7.4
	−17.7 ± 1.0 (9)	+22.4 ± 6.3	

23°C the considerable reduction in calcium flux caused by D600 is more effectively antagonized by isoprenaline than by adrenaline. It is possible that at 6°C the greater potency of adrenaline may reflect the development of an alternative pathway for the adrenergic response. In addition, calcium transmembrane flux may contribute less to the contractile response at 6°C since D600 effects a much smaller work output reduction.

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Responses of the sympathetically-innervated hepatic arterial vascular bed of the dog to intra-arterial injections of dopamine

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The hepatic arterial vascular responses to intra-arterial (i.a.) noradrenaline and adrenaline have been studied in the dog (Andrews, Hecker, Maegraith & Ritchie, 1955; Richardson & Withrington, 1976a, 1977) and have been shown to involve stimulation of both α- and β-adrenoceptors. The third naturally occurring catecholamine, dopamine, has been shown to produce both vasodilatation and vasoconstriction in the dog (Yeh, McNay & Goldberg, 1969; Sampson, Scroop & Louis, 1974), effects which may be due to stimulation of both α- and β-adrenoceptors in addition to a specific dopamine receptor. The responses of the sympathetically-innervated hepatic arterial bed of the dog to dopamine, noradrenaline and adrenaline have been compared and the receptors mediating the dopamine effects examined.

In 6 chloralose-urethane anaesthetized dogs (Richardson & Withrington, 1976c), weighing 11.9 ± 1.9 kg (mean $\pm$ s.d.), under control conditions the hepatic arterial blood flow was 183.6 ± 46.8 ml/min (mean $\pm$ s.d.), and the perfusion pressure 121.3 ± 13.4 mmHg; the calculated hepatic arterial vascular resistance (HAVR) was 0.72 ± 0.28 mmHg ml^{-1} min, or expressed in terms of the liver weights (272.0 ± 44.4 g), 1.87 ± 0.63 mmHg ml^{-1} min 100 grams.

Dopamine was injected i.a. over the range 100 ng to 1 mg to construct 8 dose-response curves in 6 experiments: each injection produced an initial increase in calculated HAVR (vasoconstriction) followed by a secondary fall in HAVR (vasodilatation). The threshold for the vasoconstrictor response was 5–50 µg, the mean dose required to double the HAVR, 6.2×10^{-7} mol, being much greater than the corresponding doses for noradrenaline, adrenaline and phenylephrine (1.1, 2.7 and 6.9×10^{-8} mol respectively). The threshold for the vasodilator response (0.1–10 µg) was lower than that for the vasoconstrictor effect, the maximum reduction in HAVR of $25.7 \pm 2.5\%$ (mean $\pm$ s.e. mean) occurred at between 10 and 200 µg in different experiments.

These responses to dopamine were similar to those to adrenaline and noradrenaline, the secondary vasodilator effects of which have been shown to be due to β-adrenoceptor activation (Richardson & Withrington, 1976b, 1977).

In three experiments, the dose-response curves for both the vasoconstrictor and vasodilator responses to dopamine were constructed before and after propranolol (0.25 mg/kg i.v.). In common with adrenaline and noradrenaline, the vasoconstrictor dose-response curve to dopamine was shifted to the left, but in contrast to adrenaline and noradrenaline, the vasodilator dopamine dose-response curve was also shifted to the left, and at one dose level of dopamine (5.3×10^{-8} mol), this potentiation was statistically significant ($P < 0.05$, paired t-test).