shown that carbenoxolone was binding at a different class of site to the other drugs.

In the present series of experiments we used a model *in vivo* to demonstrate the potentiation of warfarin anti-coagulant activity by phenylbutazone and the corresponding reduction in plasma half-life of warfarin. This model was then used to investigate any possible interaction of carbenoxolone with warfarin.

Fasted female Biorex Wistar rats (200 ± 10 g) were used in groups of six per treatment for each blood collection. All rats received 10 mg/kg warfarin sodium orally (2.5 ml/kg in distilled water) and at the same time half of the rats received subcutaneously (in 0.9% NaCl, 5 ml/kg) a dose of phenylbutazone (20 mg/kg) or carbenoxolone (40 mg/kg), i.e. at twice the molar equivalent of warfarin. Blood samples (3 ml) were obtained under ether anaesthesia at sacrifice using Hepes/citrate buffer (0.13 M trisodium citrate; 0.05 M *N* - 2 - hydroxyethylpiperazine - *N'* - 2 - ethansulphonic acid) 0.1 ml per ml of blood collected.

A one-stage prothrombin time was determined in duplicate for each rat using rabbit brain thromboplastin (Diagnostic Reagents Ltd) and warfarin concentrations were measured by spectrofluorimetry (Corn & Berberich, 1973).

No changes in prothrombin times from normal (17.0 ± 0.3 s), were seen at 8 h after warfarin treatment but at 24 h the prothrombin time was increased to 79.6 ± 1.7 s and 87.5 ± 3.5 s in the two experiments. Phenylbutazone, at a dose of twice the molar equivalent of warfarin, increased the time to 109.7 ± 7.1 s ($P < 0.005$, t-test) whilst carbenoxolone, at the twice molar equivalent, showed no significant change, 77.2 ± 3.5 seconds. Carbenoxolone and phenylbutazone had no inherent activity on prothrombin times at the dose regimens used.

The plasma half-life of warfarin, in the absence of other drugs, was found to be 8.4 and 8.5 h. Phenylbutazone reduced this to 2.3 h whilst carbenoxolone showed no change at 9.2 hours.

These findings show that carbenoxolone sodium *in vivo* does not potentiate or displace warfarin sodium and are in agreement with our earlier suggestions from studies *in vitro* (Gottfried *et al.*, 1975).

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Rat brain iso-renin

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The components of the renin-angiotensin system have been demonstrated within the brain and they have been shown to be implicated in physiological control of water and sodium balance (Fitzsimons, 1975). Recently a brain iso-renin, distinct from the renal enzyme has been demonstrated in the dog and rat brain (Ganten, Hutchinson, Schelling, Ganten & Fischer, 1976). It has also been tentatively suggested that centrally-acting hypotensive drugs may act via brain iso-renin. In order to test this hypothesis and to study in more detail the physiological role of brain iso-renin, a method has been developed which enables small amounts of enzyme present in rat brain to be measured reliably.

Rat brain extracts were incubated at 37°C in the presence of substrate prepared from nephrectomized dogs according to the method of Hass, Goldblatt, Gipson & La Vera Lewis (1966). The angiotensin produced was adsorbed on to an ion exchange resin, and subsequently eluted from the resin at the end of the incubation period. The activity of this pressor material was estimated against synthetic angiotensin II on the pithed rat blood pressure preparation. The use of (Sar^1, Ala^8)-angiotensin II, a competitive antagonist of angiotensin II (Pals, Masucci, Denning, Sipos & Fessler, 1971), completely abolished the biological activity of the material eluted from the resin. Control experiments showed that the recovery of synthetic angiotensin II amide from the resin after a 24 h incubation period under the experimental

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	