Comparative potencies of dopamine and noradrenaline on the rabbit ear artery

It has been pointed out by Iversen (1967) and Trendelenburg (1972) that the relative potencies of sympathomimetic amines on a tissue are influenced by their affinities for neuronal uptake (Uptake$_1$). In the rabbit ear artery, dopamine has been reported to possess one-fiftieth of the potency of noradrenaline (de la Lande & Harvey, 1965; Campbell & Farmer, 1968). However, in both studies the amines were injected intraluminally into the isolated artery, which was perfused in such a way that the dopamine then escaped into the extraluminal solution, i.e. the solution bathing the adventitia. It has been shown subsequently that uptake$_1$ has little influence on the intraluminal potency of noradrenaline, but a profound effect on the extraluminal potency (de la Lande & Waterson, 1967). We have now compared on the rabbit ear artery the extra- and intraluminal potencies of dopamine with those of noradrenaline, and the effect on these potencies of cocaine and chronic denervation. Relative potencies of noradrenaline and dopamine have also been determined on the ear artery helical strip.

Segments of rabbit ear arteries were perfused *in vitro* with Krebs solution in such a way that the intraluminal solution escaped without mixing with the extraluminal solution. Constriction was measured by an increase in perfusion pressure. The method has been described in detail earlier (de la Lande, Frewin & others, 1967).

Helical strips cut from freshly excised arteries were also used (de la Lande & Urquilla, 1969). The strips, approximately 3 × 0·2 cm, were placed under tension of 1 g and isotonic contraction in response to the amines was recorded via a Harvard heart/smooth muscle transducer.

In the case of perfused segments, it is not possible to obtain a satisfactory maximum response (de la Lande & Jellett, 1972). However, since the dose-response curves to the two amines were approximately parallel, the relative potencies were measured by the concentrations eliciting a standard response of 60 mm Hg. In the strips, the relative potencies were measured by the ratios of the ED50 values.

Uptake of dopamine into nerve terminals was also examined using the histochemical procedure of Falck (1962). The methods were identical to those previously described for noradrenaline (de la Lande, Hodge & others, 1970; de la Lande, Jellett & others, 1973). The principle is that arteries which are both noradrenaline-depleted and MAO-inhibited are incubated with the amine for 30 min, and the presence or absence of fluorescence at the media-adventitia border recorded. In some arteries cocaine (1 μg ml^{-1}) was present 15 min before and throughout, the period of incubation.

Other arteries were chronically denervated by the method described previously (de la Lande & Rand, 1965), using the absence of fluorescence at the media-adventitia border, when examined histochemically, as evidence that denervation was effective.

Drugs used. (−)-Noradrenaline bitartrate (Koch-Light); dopamine hydrochloride (Koch-Light); nialamide (Pfizer); reserpine (Serpasil-Ciba); ascorbic acid (Koch-Light); cocaine hydrochloride (MacFarlane-Smith).

Dopamine produced a concentration-dependent constriction of the artery when applied by either the intraluminal or extraluminal route. There was little difference between the intraluminal and extraluminal potencies, the mean ratio being close to unity (Table 1). The potencies were little affected by cocaine, or by chronic denervation (Table 2). Table 3 shows data on the relative potencies of noradrenaline and dopamine, the two amines being compared on each artery. When compared intraluminally, dopamine was approximately 140 and 150 times less potent than noradrenaline in the normal and denervated arteries, respectively. When compared extraluminally, however, dopamine was only 8 times less potent than noradrenaline in

Table 1. *Effect of denervation on the extraluminal (EL) to intraluminal (IL) ratios of dopamine and noradrenaline in the rabbit ear artery segment.*

	Normal	Denervated
Dopamine EL/IL Ratio	0·99[b] 0·87–1·12 (13)	0·96[b] 0·77–1·2 (9)
Noradrenaline EL/IL Ratio	*9·5 8·7–10·3 (17)	*1·2 1·1–1·3 (17)

* de la Lande, unpublished data (different series).

Values with same superscript do not vary significantly from one another ($P < 0.05$) (Student's *t*-test).

In this and the following tables, the values quoted are the geometric means of the potency ratios. A separate ratio was determined for each artery. The number of arteries is shown in brackets. The values immediately below the mean refer to mean ± s.e.

Table 2. *Effect of denervation and cocaine on the extraluminal and intraluminal potencies of dopamine and noradrenaline in the rabbit ear artery segment.*

	Denervated		Cocaine	
	Extraluminal	Intraluminal	Extraluminal	Intraluminal
Dopamine	1·55[c] 1·07–2·24 (6)	1·47[c] 1·01–2·14 (6)	1·2[d] 1·1–1·3 (6)	1·8[d] 1·5–2·2 (6)
Noradrenaline	26·1 23·4–29·2 (5)	1·8 1·4–2·3 (5)	*9·19 8·2–10·3 (21)	*2·12 1·85–2·34 (21)

* de la Lande, unpublished data (different series).

Values with the same superscript do not vary significantly from one another ($P < 0.05$) (Student's *t*-test).

Table 3. *Relative potencies of dopamine and noradrenaline in normal and denervated rabbit ear artery and strip.*

	Segment		Strip
	Extraluminal	Intraluminal	
Normal	7·8 6·7–9·0 (9)	142[a] 102–198 (5)	32·9[a] 23·6–45·8 (12)
Denervated	110[a] 90–134 (5)	150[a] 118–189 (5)	—

Values with the same superscript do not vary significantly from one another ($P < 0.05$) (Student's *t*-test).

In the normal arteries, the means ± s.e. of the equieffective concentrations which gave a response of 60 mm Hg were: extra- and intra-luminal dopamine 13 ± 2 and 14 ± 4 × 10^{-9}M respectively, and extra- and intra-luminal noradrenaline 1·9 ± 0·5 and 0·087 ± 0·017 × 10^{-9}M respectively. The ED50 in strips was 16 ± 4 × 10^{-9}M for dopamine, and 0·83 ± 0·31 × 10^{-9}M for noradrenaline.

normal arteries, but the ratio was increased to 110 in chronic denervated arteries. In strips, the dose-response curves were approximately parallel and there was no significant difference between the maximum responses. The potency of dopamine was 1/33rd that of noradrenaline.

The histochemical studies showed that incubation with extraluminal dopamine (at concentrations greater than 0·01 μg ml^{-1}) led to the appearance of marked fluorescence at the media-adventitia border. Cocaine (1 μg ml^{-1}) prevented the appearance

of fluorescence when dopamine was present in concentrations of 0·01–0·3 μg ml^{-1}, but did not do so when dopamine was present in concentrations of 1–3 μg ml^{-1}. The latter concentrations were selected to be in the lower range of the concentrations which caused vasoconstriction on most preparations.

The profound effects of cocaine and chronic denervation on extraluminal noradrenaline sensitivity compared with the minor effects on intraluminal noradrenaline sensitivity have been described and interpreted as evidence that Uptake$_1$ represents a considerable site of loss of extraluminal noradrenaline, but not of intraluminal noradrenaline, as the amine diffuses from the surface of the artery to the receptors in the smooth muscle (de la Lande & Waterson, 1967; de la Lande & others, 1970). In contrast, the pharmacological effects of dopamine are consistent with failure of Uptake$_1$ to influence either its extraluminal or intraluminal potency. Thus, in contrast to noradrenaline (i) the ratio of the intraluminal and extraluminal potencies is approximately one, and (ii) neither potency is affected to a significant degree by cocaine or chronic denervation. The relatively minor role of Uptake$_1$ at the concentration required for pharmacological activity probably reflects saturation of uptake, as suggested by other workers (Langer & Trendelenburg, 1969), since our results show that in this artery cocaine can unequivocally prevent the appearance of monoamine fluorescence in the region of the nerve terminals only when the concentration of dopamine is considerably below the concentration which elicits vasoconstriction.

It is noteworthy that the mean ratio of the relative potencies of intraluminal noradrenaline and dopamine in untreated arteries (142) is very much greater than the ratio of the extraluminal potencies (7·8). However, the ratio of the extraluminal potencies becomes approximately equal to that of the intraluminal potencies when these are estimated under conditions where Uptake$_1$ is prevented or eliminated. Hence the intraluminal ratios provide a useful index of relative potencies uncomplicated by Uptake$_1$. The fact that the ratio is 2–3 fold greater than those estimated previously probably reflects the fact that in the single cannulated preparation, following intraluminal administration, the drug is present in the extraluminal fluid, and under this condition dopamine, but not noradrenaline, could further contribute to the vasoconstrictor response. The observations on the untreated strip preparation indicate a ratio which is approximately the geometric mean of the two ratios (intraluminal and extraluminal) prevailing in the untreated segment. Clearly, inhibition of uptake in the strip preparation, and presumably in other types of arterial strip preparations, would tend to underestimate the maximum contribution which Uptake$_1$ can exert on the relative activities of dopamine and noradrenaline.

A brief account of this work was presented at the November 1970 meeting of the Australasian Society of Clinical and Experimental Pharmacologists.

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July 17, 1973

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Appearance in cat cerebrospinal fluid of radiolabelled metabolites following intraventricular injection of [^{3}H]choline

The metabolism of choline in brain tissue has been observed following intracerebral and intravenous administration (Ansell & Spanner, 1968; Diamond, 1971; Barker, Dowdall & Whittaker, 1972; Dowdall, Barker & Whittaker, 1972). We have injected [^{3}H]choline into the lateral cerebral ventricles of the cat and studied the appearance of radiolabelled metabolites in the effluent obtained during ventriculo-aqueductal perfusion.

Cats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}) and mounted in a stereotaxic instrument. After cannulation of the right lateral ventricle and aqueduct, perfusion with artificial cerebrospinal fluid (csf) (in mM: Ca, 1·3; Na, 151·1, Mg, 0·9; K, 2·6; HCO_3, 21·0; HPO_4, 2·5; glucose, 3·4), thoroughly gassed with a mixture of CO_2 and oxygen, was begun at the rate of 100 μl min^{-1} with a Technicon auto-proportioning pump.

After 30 min, the perfusion was stopped, the outflow occluded and [^{3}H]choline chloride (30 μl; 1 μCi μl^{-1}; 17 Ci $mmol^{-1}$, Amersham Searle) injected. After 10 min, the perfusion was begun again with csf containing 1×10^{-4}M physostigmine sulphate. The effluent was collected at 5 min intervals, placed on ice in tubes containing 50 μl of 1×10^{-3}M neostigmine sulphate in acetate buffer (0·01 M), and either assayed immediately or frozen at −20°.

Aliquots were placed in Triton-toluene phosphor (TTP) and counted with a Packard Tri-carb liquid scintillation counter having a tritium counting efficiency of 20%. To separate lipids from water-soluble metabolites, 200 μl samples were mixed with 10 vol of a chloroform–methanol solution (2:1), capped, thoroughly agitated and allowed to stand for 12 h at 4°. The phases were then separated, the chloroform–methanol portion evaporated to dryness, TTP added and the samples counted. Water soluble metabolites were separated by high voltage electrophoresis (HVE). Authentic standards of choline, acetylcholine, betaine, and phosphorylcholine were run simultaneously and visualized with platinum-iodine reagent.

Fig. 1a illustrates the mean two phased decay curve obtained in 4 cats following the initiation of ventricular perfusion after injection of [^{3}H]choline chloride. Given that the effective volume of the cat ventricular system is about 10 ml, the results suggest that approximately 68% of the injected radioactivity remained in the ventricular space after 10 min. This rapid clearance of choline from the ventricular space was also observed by Aquilonius & Winbladh (1972). While the principal constituents in the early periods of the perfusion were water soluble (see Fig. 1b), there was a progressive increase in the relative proportion of lipid metabolites. At 240 min, the amount of recovered label incorporated into water-soluble metabolites and lipids was virtually identical.