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Temperature dependence of catecholamine depletion by reserpine in the heart of the toad *(Bufo marinus)*

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Summary

1. The catecholamines in toad ventricle were adrenaline (90%) and noradrenaline (10%); there was no dopamine.

2. Phenoxybenzamine and tyramine stimulated the isolated heart and reduced the catecholamine content.

3. Reserpine treatment of toads kept at 20° C did not affect the adrenaline but reduced the noradrenaline content of the ventricle.

4. At 37° C, reserpine caused depletion of both adrenaline and noradrenaline, and the stimulant actions of phenoxybenzamine and tyramine were lost.

Introduction

Administration of reserpine leads to depletion of the catecholamine content of mammalian tissues. However, reserpine does not cause depletion of the catecholamine content of toad tissue (Nayler, 1963; Boyd, Burnstock & Rogers, 1964). In investigating the lack of susceptibility of tissue catecholamines of the toad to the depleting action of reserpine, observations were made with other types of drugs which release catecholamines from mammalian tissues; those chosen were tyramine (Burn & Rand, 1958) and phenoxybenzamine (Schapiro, 1958; Chang & Fearn, 1969).

Methods

Freshly caught toads (*Bufo marinus*) were obtained from Queensland and brought to Melbourne by air-freight. Reserpine (Serpasil) was given by intraperitoneal injection. Body temperature was measured by inserting the probe of an Electric Universal Thermometer into the stomach through the mouth.

After pithing, the heart was removed and a cannula was inserted into the ventricular chamber through the truncus arteriosus, after Straub's method. The Ringers solution had the following composition (in mmol/l.): NaCl, 128; KCl, 2·7; Na_2HPO_4, 0·8; $CaCl_2$, 1·1; $MgSO_4$, 1·0; glucose, 10 and was used at room temperature (about 20° C). The contractions of the ventricle were recorded on a kymograph with an isotonic frontal writing lever. Each preparation was allowed to beat

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for 15 min in contact with Ringers solution ; in some experiments the solution contained 1 μg/ml of tyramine hydrochloride or 1 μg/ml of phenoxybenzamine hydrochloride.

The ventricular catecholamine contents were assayed in hearts which had been set up as Straub preparations and on hearts freshly removed from pithed toads. The ventricle was dried by blotting, weighed on a torsion balance and crushed with aluminium tongs previously cooled in liquid air. The frozen wafers of muscle were quickly ground with a pestle and mortar which had also been precooled with liquid air. The catecholamines were extracted with 0·01 N HCl (Shore & Olin, 1958) and separated by paper chromatography (Euler & Hamberg, 1949). The portions of the chromatogram containing each catecholamine were identified under ultraviolet light. They were cut out and the amines were extracted into 25 mM NaH_2PO_4 to which 1 mg of ascorbic acid was added. After centrifugation at 200 r.p.m. at 0° C, an aliquot of supernatant was taken. The catecholamines were assayed using the pressor responses of the pithed rat (Brown & Gillespie, 1957) and the reduction of contractile responses of the rat uterus (Harvey & Pennefather, 1962). Recovery was in the range of 80–90% ; no corrections were applied.

Results

Catecholamine content of the toad cardiac ventricle and effects of tyramine and phenoxybenzamine

Chromatograms of extracts of toad ventricle contained spots having R_F values corresponding to those of adrenaline and noradrenaline ; there were no traces of spots corresponding to dopamine.

Ventricles from Straub preparations of the toad's heart had a mean content of 1·4 μg/g of adrenaline and 0·15 μg/g of noradrenaline (Table 1).

The amplitude and sometimes the rate of beating was increased when the Ringers solution contained 1 μg/ml of tyramine or 1 μg/ml of phenoxybenzamine, and the adrenaline and noradrenaline contents of the ventricle were significantly reduced after 15 min (Table 1).

Effects of reserpine on catecholamine content and responses to tyramine and phenoxybenzamine

At the normal laboratory ambient temperature of 18–22° C, the body temperature of the toads was about 23° C. Administration of reserpine (3 or 10 mg/kg daily

TABLE 1. *Effects of tyramine and phenoxybenzamine on catecholamine content of toal isolated heart*

Treatment	Mean content ± S.E. of mean in μg/g tissue		Number of observations
	Adrenaline	Noradrenaline	
Ringers solution	1·4 ±0·15	0·15±0·04	5
Tyramine (1 μg/ml)	0·65±0·24*	<0·050†	5
Phenoxybenzamine (1 μg/ml)	0·71±0·12*	<0·045†	5

* These means were significantly different from the control with $P<0·001$ (t test). † With some samples, the catecholamine content was at or below the threshold of the assay method, consequently the standard error has not been calculated.

for 3 days) did not result in any significant reduction of the adrenaline content of ventricles ; however, the noradrenaline content was reduced (Table 2). The larger dose caused some deaths. At an ambient temperature of 37° C, which resulted in an increase of body temperature to 28° C, administration of reserpine (3 mg/kg daily for 3 days) caused almost complete disappearance of the adrenaline and noradrenaline contents of the cardiac ventricle (Table 2).

The responses to tyramine or phenoxybenzamine of Straub preparations taken from toads treated with reserpine and maintained in an environmental temperature of 18–22° C did not differ from those taken from untreated toads maintained at the same environmental temperature or at 37° C. However, preparations from toads treated with reserpine and maintained at 37° C failed to respond to tyramine or phenoxybenzamine.

Discussion

The main catecholamine in the cardiac ventricle of the toad is adrenaline, which accounts for about 90% of the total content ; the remainder is noradrenaline and there is no dopamine. These findings agree with those reported for the frog (Ostlund, 1954 ; Falck, Häggendal & Owman, 1963).

Reserpine depletes adrenaline and noradrenaline stores in mammalian heart (Bertler, Carlsson & Rosengren, 1956 ; Muscholl, 1959). However, in toads kept at 18–22° C the noradrenaline content of the heart was reduced by reserpine whereas the content of the main catecholamine, adrenaline, was not affected. This was so even with doses of reserpine that caused some deaths. That the adrenaline stores in the toad are not sensitive to depletion by reserpine under similar conditions has also been reported by Nayler (1963) and Boyd *et al.* (1964). The latter workers reported that as high a dose as 50 mg/kg of reserpine given for 3 days did not affect the adrenaline stores in the toad colon. It is possible that the mechanism involved in the binding of adrenaline in tissues of the toad differs from that in mammalian tissues. However, when toads were kept at an environmental temperature of 37° C, which raised their body temperature to 28° C, reserpine caused a marked depletion of cardiac catecholamines, as it does in mammals, which suggests that there is no fundamental difference between mammalian and toad adrenergic tissues, but that the reactions leading to loss of catecholamines are temperature dependent. Other

TABLE 2. *Effect of environmental temperature on catecholamine depleting action of reserpine in the toad*

Drug treatment	Environmental temperature	Catecholamines in ventricle: mean ± S.E. of mean in μg/g tissue		Number of observations
		Adrenaline	Noradrenaline	
None	37° C	1·6±0·07	0·11±0·05	4
Reserpine (3 mg/kg daily for 3 days)	18–22° C	1·3±0·11	<0·05*	5
Reserpine (3 mg/kg daily for 3 days)	37° C	<0·24*	<0·001*	5
Reserpine (10 mg/kg daily for 3 days)	18–22° C	1·1±0·11	<0·045*	5

* The catecholamine content of some samples was at or below the threshold of the assay method, consequently the S.E. was not calculated.

drugs which act on cardiac catecholamine stores have the same actions in toad and mammalian hearts. Thus, tyramine caused a loss of adrenaline and noradrenaline from the toad heart and there was an increase in the amplitude and sometimes in the rate of beating. This cardiac stimulation is in accord with the observations made on the mammalian heart with tyramine (Lindmar & Muscholl, 1961 ; Davey & Farmer, 1963). Similarly, when the toad heart was exposed to phenoxybenzamine there was a considerable reduction in adrenaline and noradrenaline in the heart and the release of these catecholamines probably explains the stimulant actions of phenoxybenzamine on the isolated toad heart. In isolated perfused rat heart, phenoxybenzamine has sympathomimetic actions (Chang, 1968) and reduces catecholamine stores (Chang & Fearn, 1969). Tyramine and phenoxybenzamine did not cause the usual stimulation of the isolated toad heart when the catecholamine content had been depleted by a combination of reserpine and high environmental temperature.

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