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NO EVIDENCE FOR REVERSE TRANS-SYNAPTIC REGULATION OF NEURONAL UPTAKE BY β-ADRENOCEPTORS IN KITTEN ATRIA

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Abstract—Kitten atria incubated with [^{3}H]noradrenaline, 1.18×10^{-7} M for 10 min or 3×10^{-9} M for 30 min, actively accumulated the amine. Final tissue tritium concentrations were 2–4-fold and 8–12-fold higher, respectively, than those of the incubation fluid. Uptake was consistently greater in right than in left atria. The β_1-adrenoceptor antagonist, practolol, 10^{-6} or 10^{-5} M, and the β_1- and β_2-adrenoceptor antagonist, propranolol, 5×10^{-8}, 5×10^{-7} or 5×10^{-6} M, did not affect noradrenaline uptake. Reverse trans-synaptic regulation of neuronal noradrenaline uptake by β-adrenoceptors therefore does not appear to operate in kitten atria as has been reported for rat atria and other tissues.

Manukhin, Volina and Melenteva [1] have suggested that post-synaptic adrenoceptors regulate neuronal uptake of noradrenaline such that, if activity at adrenoceptors is decreased, there is a compensatory increase in noradrenaline uptake by adrenergic neurones. They based this hypothesis on the observations that in isolated rat tissues which contain predominantly α-adrenoceptors post-junctionally, phentolamine increased and phenylephrine decreased neuronal uptake of noradrenaline, while in those with mainly β-adrenoceptors, propranolol increased and isoprenaline decreased uptake. They also observed qualitatively similar but smaller changes with propranolol and isoprenaline in tissues where the dominant adrenoceptors were of the α-type and suggested that these effects were due to the co-existence of a sub-population of β-adrenoceptors within the tissues. In later work, the above authors [2] looked for regulatory humoral factors that might be involved by carrying out experiments of the recipient–donor type in the presence and absence of cycloheximide. They concluded from their results that reverse trans-synaptic regulation of neuronal noradrenaline uptake, whether it involved stimulation or inhibition of uptake, was due to release of humoral factors which were in turn dependent on *de novo* protein synthesis.

It has been known for many years that both transportable and non-transportable adrenoceptor agonists can inhibit the neuronal uptake of noradrenaline [3]. However, there is little evidence from other sources that adrenoceptor antagonists stimulate neuronal uptake. It is, in fact, generally accepted that in concentrations greater than those required to produce a substantial adrenoceptor blockade, they inhibit noradrenaline uptake [4]. Furthermore, there is no correlation between inhibition of either pre- or post-synaptic adrenoceptors and blockade of neuronal uptake [5, 6]. The most marked change in neuronal uptake observed by the Russian workers [1, 2] occurred in rat atria treated with propranolol. It was decided to see whether a similar stimulatory effect on uptake could be observed in another species. The kitten was chosen, since both neuronal and extraneuronal uptake have been previously characterized in atria from this species [7, 8].

MATERIALS AND METHODS

Kittens of either sex, weighing 500 to 1000 g, were anaesthetized with ether and the heart removed and continuously perfused with cold physiological solution gassed with 5% CO_2 in O_2. The physiological solution was either McEwen solution [9] to which EDTA 6.7×10^{-5} M and ascorbic acid 1.14×10^{-4} M had been added or was the Tyrode solution used by Manukhin and Volina [2]. The left and right atria were removed from the heart and cut transversely into halves which were then randomly allocated to control or treated groups. Atrial halves were placed under a resting tension of 2 g in organ baths containing 2 ml oxygenated physiological solution at 37° and allowed to equilibrate for 1 hr. The bath fluid was changed and the tissue incubated for 30 min with a β-adrenoceptor antagonist or an equivalent volume of saline. [^{3}H]Noradrenaline 3×10^{-9} M or 1.18×10^{-7} M (0.0134 μCi/ml or 0.107 μCi/ml respectively) or [^{3}H]sorbitol 6.6×10^{-11} M (0.112 μCi/ml) plus unlabelled noradrenaline (3×10^{-9} M or 1.18×10^{-7} M) was then injected into the bath. Atria were removed 10, 30 or 40 min after addition of tritium. In some experiments atria were then washed 5 times at 1 min intervals with 5 ml ice cold physiological saline. They were blotted dry with Whatman no. 1 filter paper, placed in scintillation vials and weighed. Tissues were either digested with 1 ml NaOH (1 M) at 75° for 8 hr or were extracted with 1 ml absolute ethanol at room

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Table 1. Uptake of [^{3}H]noradrenaline by kitten atria following 10 min incubation with amine (1.18×10^{-7} M)

Treatment		[^{3}H]Noradrenaline uptake (nmoles/g wet wt tissue) Left atria	Right atria
None (16)*		0.31 ± 0.01†	0.43 ± 0.02‡
Practolol	10^{-6} M (10)	0.32 ± 0.02	0.49 ± 0.05‡
Practolol	10^{-5} M (10)	0.33 ± 0.03	0.40 ± 0.05
None (12)		0.28 ± 0.02	0.43 ± 0.03‡
Propranolol	5×10^{-8} M (14)	0.27 ± 0.02	0.40 ± 0.03‡
None (8)		0.33 ± 0.02	0.50 ± 0.05‡
Propranolol	5×10^{-7} M (10)	0.31 ± 0.01	0.50 ± 0.03‡
None (10)		0.34 ± 0.02	0.51 ± 0.04‡
Propranolol	5×10^{-6} M (10)	0.29 ± 0.01	0.44 ± 0.05‡

* Figures in parentheses indicate number of left or right atrial samples tested.
† Values are given ± S.E.M.
‡ Value for right atria is significantly higher than that for corresponding left atria, $P < 0.01$ (Student's non-paired t-test). There is no significant difference between values for treated and untreated tissues.

temperature for 18 hr prior to NaOH digestion. Alkaline digests were neutralized with 0.1 ml HCl (12 M) and 15 ml of a 2:1 toluene:Triton X-100 mixture containing 0.4% PPO was added. Alcoholic extracts were mixed with 10 ml toluene containing 0.4% PPO and 0.01% POPOP. Radioactivity was determined in a Packard liquid scintillation spectrometer, model 3390. A 1 ml aliquot of each bath solution was taken for estimation of radioactivity. Quench correction was employed using the automatic external standardization facility.

The drugs used were noradrenaline hydrochloride (Sigma Chem. Co.), practolol and propranolol hydrochloride (Imperial Chemical Industries), [1-^{3}H]-(+)sorbitol (Radiochemical Centre, Amersham) and [7-^{3}H]-(−)noradrenaline (New England Nuclear).

RESULTS

Atrial samples incubated with [^{3}H]noradrenaline (1.18×10^{-7} M) in McEwen solution for 10 min and then blotted dry contained 2–4-fold greater concentrations of tritium than the incubation fluid, thus indicating active accumulation of amine (Table 1). Accumulation was significantly greater in right than in left atria. Estimations of tissue tritium levels underestimated the cellular concentrations of noradrenaline and its metabolites since at least 42–47 per cent of the tissue weight was found to be extracellular fluid when [^{3}H]sorbitol was used as an extracellular marker (Table 2). The extracellular fluid volume in atria was, in fact, greater than this estimate since sorbitol spaces in the atria at 10 min were only 86 and 80 per cent of the corresponding values obtained for left and right atria respectively at 40 min. Propranolol (5×10^{-7} M) did not affect the distribution of sorbitol. In atria incubated with [^{3}H]noradrenaline neither the β_1-adrenoceptor antagonist practolol (10^{-6} and 10^{-5} M) nor the non-selective β-adrenoceptor antagonist propranolol (5×10^{-8}, 5×10^{-7} and 5×10^{-6} M) significantly increased or decreased tissue tritium levels (Table 1).

In view of the negative results obtained above with practolol and propranolol, further experiments were carried out using the experimental conditions described by Manukhin and Volina [2], i.e. a lower concentration of [^{3}H]noradrenaline (3×10^{-9} M) in a Tyrode solution was used for a 30 min rather than a 10 min incubation. Atrial samples were washed 5 times after the incubation before alcoholic extraction

Table 2. Mean sorbitol spaces for kitten atria following incubation with noradrenaline (1.18×10^{-7} M)

Time (min)	Treatment		Sorbitol spaces (ml/g) Left atria	Right atria
10	None (8)*		0.44 ± 0.01†	0.46 ± 0.02
	Propranolol	5×10^{-7} M (8)	0.42 ± 0.01	0.47 ± 0.01‡
40	None (8)		0.52 ± 0.02	0.51 ± 0.01
	Propranolol	5×10^{-7} M (8)	0.53 ± 0.02	0.50 ± 0.02

* Figures in parentheses indicate number of left or right atrial samples tested.
† Values are given ± S.E.M.
‡ Value for right atria is significantly higher than that for corresponding left atria, $P < 0.05$ (Student's non-paired t-test). There is no significant difference between values for treated and untreated tissues.

Table 3. Uptake of [^{3}H]noradrenaline by kitten atria following 30 min incubation with amine (3×10^{-9} M)

Treatment		[^{3}H]Noradrenaline uptake (pmoles/g wet wt tissue) Left atria	Right atria
None (9)*		27 ± 2†	44 ± 5‡
Propranolol	5×10^{-7} M (9)	24 ± 2	39 ± 2‡
None (14)¶		24 ± 2	35 ± 3‡
Propranolol	5×10^{-7} M (14)	25 ± 2	37 ± 3‡

* Figures in parentheses indicate number of left or right atrial samples tested.

† Values are given ± S.E.M.

‡ Value for right atria is significantly higher than that for corresponding left atria $P < 0.01$ (Student's non-paired t test). There is no significant difference between values for treated and untreated tissues.

¶ Following incubation with [^{3}H]noradrenaline, atria were washed 5 times at 1 min intervals with 5 ml cold Tyrode solution.

and counting of the extracts and tissue residues. It was found that the amount of tritium extracted by 1 ml alcohol differed markedly between samples but correlated with the weight of the tissue. The correlation coefficient for left atria of mean weight 79 ± 5 mg ($n = 28$) was 0.88 ($P < 0.001$) and for right atria of mean weight 66 ± 6 mg ($n = 28$) was 0.84 ($P < 0.001$). Tissue tritium levels obtained by alcoholic extraction were thus meaningless. Total tissue values were therefore calculated from the sum of the counts for the alcoholic extract and the residual tissue, since changes in neuronal uptake are reflected by changes in total tissue tritium concentrations [7]. Such values were found to be 8-fold higher in left atria and 12-fold higher in right atria than those of the incubation fluid, again indicating active accumulation of amine (Table 3). Similar values were obtained if the washing procedure was omitted, thus confirming that most of the tritium was located intracellularly since washing reduced the [^{3}H]sorbitol content of the extracellular fluid by 70 per cent (Table 4). Propranolol (5×10^{-7} M) failed to alter sorbitol spaces or tritium uptake in atria incubated with [^{3}H]noradrenaline irrespective of whether samples were washed five times or simply blotted dry (Tables 3 and 4).

DISCUSSION

Following incubation of kitten atria with [^{3}H]noradrenaline (1.18×10^{-7} M) there is an active uptake of the amine (and its metabolites). This result confirms observations made in a previous study [7]. Such uptake is mainly neuronal since it is temperature sensitive and inhibited by imipramine, cocaine and metaraminol but not by the extraneuronal uptake inhibitor cortisol [7, 8]. Active accumulation of amine was even more marked with the lower concentration of noradrenaline (3×10^{-9} M) tested. At either amine concentration, uptake was consistently greater in right than in left atria again substantiating earlier findings [7]. However, neither the selective β_1-adrenoceptor antagonist practolol (10^{-6} and 10^{-5} M) nor the non-selective β-adrenoceptor antagonist propranolol (5×10^{-8}, 5×10^{-7} and 5×10^{-6} M) had any effect on neuronal uptake. The concentrations of antagonists tested were in the ranges required to produce a substantial blockade of β-adrenoceptors. For example in kitten atria the reported values for negative log K_B are for practolol on rate 6.55 and 6.4 [10, 11] and for propranolol on rate and force 8.5 and 8.7 respectively [12].

A possible explanation for the discrepancy between the present results and those reported in the rat [1, 2] is that there is a species difference in response of neuronal uptake to β-adrenoceptor antagonists. However, this seems unlikely since propranolol (5×10^{-7} M) did not affect amine uptake when we repeated the experiments with rat atria (40–50 mg) and uteri (45–50 mg) using [^{3}H]noradrenaline (3×10^{-9} M), and the same experimental conditions as those described by Manukhin and Volina [2]. For atria, alcoholic extraction gave values for noradrenaline uptake of 39.6 ± 2.3 ($n = 10$) and 33.1 ± 2.9 ($n = 9$) pmoles/g wet weight tissue for control and treated atria respectively, while the corresponding total uptake values were 65.6 ± 4.1 and 59.7 ± 3.6 pmoles/g. For uteri, alcoholic extraction values were 7.1 ± 0.5 ($n = 8$) and 6.5 ± 1.1. ($n = 7$) pmoles/g for control and treated uteri while the corresponding total values were 13.0 ± 1.1 and 11.8 ± 1.8 pmoles/g. It should be noted that in these experiments a narrow range of tissue weights was used in view of our finding that alcoholic extraction of tritium was very dependent on tissue weight.

Table 4. Mean sorbitol spaces for kitten atria following incubation with noradrenaline (3×10^{-9} M)

Time (min)			Sorbitol spaces (ml/g) Left atria	Right atria
30	None (8)*		0.45 ± 0.01†	0.47 ± 0.01
	Propranolol	5×10^{-7} M (8)	0.50 ± 0.01	0.49 ± 0.01
40	None (4)		0.48 ± 0.03	0.49 ± 0.03
	Propranolol	5×10^{-7} M (4)	0.50 ± 0.03	0.48 ± 0.04
30	None (8)‡		0.13 ± 0.01	0.12 ± 0.01
	Propranolol	5×10^{-7} M (8)	0.11 ± 0.02	0.12 ± 0.01

* Figures in parentheses indicate number of left or right atrial samples tested.

† Values are given ± S.E.M.

‡ Following incubation with [^{3}H]noradrenaline, atria were washed 5 times at 1 min intervals with 5 ml cold Tyrode solution.

The only major difference in experimental technique between the present study and that of Manukhin and Volina [2] is that tissues were maintained under a fixed tension throughout incubation with tracer. This was done in order to keep the surface area of the tissue exposed to the incubation fluid as constant as possible. However, it seems improbable that this could account for us finding no change in amine uptake in rat atria treated with propranolol (5×10^{-7} M) while Manukhin and Volina [2] found nearly a three-fold increase.

The present results obtained using kitten and rat atria agree with those for rabbit hearts [5] where perfusion with seven β-adrenoceptor antagonists at a concentration of 4×10^{-7} M did not markedly change noradrenaline uptake. Higher concentrations (2×10^{-6} M–5×10^{-5} M) either did not change or reduced uptake. This inhibitory effect of high concentrations of β-receptor antagonists on uptake has also been observed in rat hearts and other tissues [4], and is unrelated to β-adrenoceptor antagonism [5, 6]. There is thus no evidence to support the concept that β-adrenoceptors in the heart exert a modulatory role with respect to neuronal uptake of noradrenaline [1, 2].

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