Maintenance of noradrenaline in neuronal cell bodies and terminals: effect of frequency of stimulation

Upon the arrival of action potentials in noradrenergic nerve terminals noradrenaline is released (Brown, 1960; Haefely, Hürlimann & Thoenen, 1965), much of the released noradrenaline is retrieved (Blakeley, Brown & Geffen, 1964), and noradrenaline synthesis is accelerated (Alousi & Weiner, 1966). The results of these studies suggest that steady state levels of noradrenaline in the nerve terminals are maintained by local synthesis and by reuptake. The relative importance of these two processes in resting and active neurons remains controversial (Malmfors, 1964; Hedqvist and Stjarne, 1969). For example, in nerve terminals, synthesis of noradrenaline is believed to be of prime importance at low frequencies of stimulation while reuptake of this amine predominates at high frequencies (Bhagat and Friedman, 1969). On the other hand, in noradrenergic nerve cell bodies noradrenaline stores appear to be maintained by synthesis independent of neuronal activity (Moore & Bhatnagar, 1970). We have examined further the roles of synthesis and uptake in the maintenance of noradrenaline stores in both cell bodies and terminals of noradrenergic nerves during different frequencies of stimulation.

Experiments were conducted in 1·6–3·5 kg cats anaesthetized with Dial-Urethane. Superior cervical ganglia represented the cell bodies, and salivary glands and nictitating membranes the terminals of peripheral noradrenergic neurons. Preganglionic fibres were decentralized and stimulated unilaterally at low and high frequencies of stimulation (2 or 10 Hz for 30 s/min for 3 h). The upper limit of "physiological" frequency of impulses is thought to be 10 Hz (Folkow, Häggendal & Lisander, 1967). The viability of the ganglionic transmission was monitored by recording contractions of the nictitating membranes during preganglionic stimulation. Synthesis of noradrenaline was inhibited by α-methyltyrosine (Spector, Sjoerdsma & Udenfriend, 1965) infused through both common carotid arteries at the rate of 0·1 mg/min for 3 h. This dosage schedule had been found by us to block the synthesis of [^{14}C]noradrenaline from [^{14}C]tyrosine in ganglia, salivary glands and nictitating membranes. Control animals received infusions of 0·9% NaCl. Saline and α-methyltyrosine infusions were begun at the start of the preganglionic stimulation. Re-uptake of noradrenaline was blocked by intravenous injections of desipramine, 2 mg/kg of which were administered at the start of stimulation and another 1 mg/kg 2 h later; a dose found by us to block the uptake of [^{3}H]noradrenaline in submaxillary salivary glands and nictitating membranes but not in ganglia. At the end of the experiment, tissues were removed and analysed for noradrenaline. Submaxillary salivary glands (frozen in liquid nitrogen and pulverized) and ganglia were homogenized in 0·4N $HClO_4$. Nictitating membranes with orbital attachments were cut into small pieces and allowed to stand in 0·4N $HClO_4$ overnight. The noradrenaline in perchloric acid extracts was isolated by alumina adsorption and analysed by the trihydroxyindole method as described by Moore & Rech (1967).

The effects of α-methyltyrosine, desipramine, and different frequencies of stimulation on the noradrenaline contents of superior cervical ganglia, salivary glands and nictitating membranes are summarized in Table 1.

We have previously reported that in unstimulated preparations α-methyltyrosine reduces the noradrenaline concentrations in cell bodies but not in terminals, while desipramine has no effect on the noradrenaline content in either cell bodies or terminals (Bhatnagar & Moore, 1970). Although the present experiments were not specifically designed to compare drug effects in non-stimulated preparations, the same pattern was apparent. That is, when the effects of drugs in all non-stimulated tissues

Table 1. *Effects of α-methyltyrosine, desipramine and electrical stimulation at 2 and* 10 Hz *on tissue noradrenaline contents.*

Treatment	Frequency of stimulation (Hz)	Superior cervical ganglia (μg/g)			Submaxillary salivary glands (μg/g)			Nictitating membranes (μg/membrane)		
		N	S	S/N × 100	N	S	S/N × 100	N	S	S/N × 100
Saline	2	5·96 ± 0·97	6·92 ± 0·93	116	1·27 ± 0·16	1·02 ± 0·09	81	1·37 ± 0·22	1·28 ± 0·25	93
	10	8·25 ± 0·81	7·50 ± 0·34	91	1·70 ± 0·30	0·72 ± 0·03	42†	1·22 ± 0·08	0·90 ± 0·09	74†
α-Methyltyrosine	2	3·32 ± 0·73	3·50 ± 0·34	105	1·89 ± 0·23	1·07 ± 0·17	57*†	1·01 ± 0·17	0·76 ± 0·17	75
	10	3·75 ± 0·39	3·37 ± 0·52	90	1·29 ± 0·10	0·16 ± 0·25	12*†	1·08 ± 0·13	0·52 ± 0·03	48*†
Desmethylimipramine	2	6·59 ± 1·14	6·40 ± 1·36	97	1·47 ± 0·31	1·04 ± 0·22	71†	1·24 ± 0·12	1·04 ± 0·18	84
	10	9·26 ± 1·08	8·38 ± 1·00	90	1·63 ± 0·16	0·39 ± 0·05	24*†	1·63 ± 0·27	0·98 ± 0·19	60*†
α-Methyltyrosine and desmethylimipramine	2	2·57 ± 0·54	2·60 ± 0·77	101	1·30 ± 0·17	0·58 ± 0·08	45*†	0·92 ± 0·12	0·80 ± 0·18	87
	10	2·06 ± 0·12	2·82 ± 0·66	137	1·70 ± 0·18	0·04 ± 0·00	2*†	1·26 ± 0·11	0·47 ± 0·10	37*†

Values in non-stimulated (N) and in stimulated (S) tissues represent the mean content of noradrenaline ±1 s.e. determined in 3–7 separate experiments.
*—Significantly different from corresponding saline controls ($P < 0.05$).
†—Significantly different from 100% ($P < 0.05$).

were collated it was clear that α-methyltyrosine, but not desipramine, significantly reduced the noradrenaline content of ganglia (saline 6·88 ± 0·68 μg/g; α-methyltyrosine, 3·49 ± 0·45 μg/g; desipramine, 8·08 ± 0·87 μg/g). On the other hand, neither desipramine nor α-methyltyrosine significantly altered noradrenaline contents in salivary glands and nictitating membranes.

Since there was much less variation in the tissue concentration of noradrenaline between the right and left sides of the same cat than there was between cats, experiments on the effects of stimulation were designed so that the contralateral tissues of the same cat served as controls. Preganglionic stimulation alone or in the presence of α-methyltyrosine or desipramine, or both, did not alter the noradrenaline content of ganglia. That is, the noradrenaline concentrations in stimulated ganglia expressed as a percentage of noradrenaline in non-stimulated ganglia were not statistically different from 100%. Thus, noradrenaline in ganglia is maintained by synthesis independent of the frequency of neuronal activity. In contrast to the ganglia, preganglionic stimulation alone reduced noradrenaline concentrations in salivary glands; stimulation at 2 and 10 Hz reduced noradrenaline content to 81 and 42% of the corresponding unstimulated gland. α-Methyltyrosine caused a further reduction in the stimulus-induced decline of noradrenaline at both 2 and 10 Hz (to 57 and 12% respectively), whereas desipramine increased the depletion of noradrenaline only at the higher frequency of stimulation. The combination of desipramine and α-methyltyrosine enhanced the noradrenaline depletion at 2 Hz and caused an almost total depletion at 10 Hz. The effects of preganglionic stimulation, desipramine and α-methyltyrosine in nictitating membranes were qualitatively similar to those seen in salivary glands with the effects being significant only at the higher frequency of stimulation and the changes in noradrenaline concentrations being less pronounced. Thus, in nerve terminals, synthesis partially maintains noradrenaline concentrations during high and low frequencies of stimulation. Reuptake, on the other hand, appears to play a significant role only at higher frequencies of stimulation.

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