Short communications

Differential labelling of intraneuronal noradrenaline stores with different concentrations of (−)-^{3}H-noradrenaline

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Isolated preparations of the rabbit vas deferens were incubated with (−)-^{3}H-noradrenaline. A preferential release of ^{3}H-noradrenaline was demonstrated after electrical stimulation of the intramural nerves in those tissues labelled with 10 ng/ml ^{3}H-noradrenaline. There was no preferential release of ^{3}H-noradrenaline when the vasa were initially labelled with 100 ng/ml ^{3}H-noradrenaline. It is concluded that low concentrations of ^{3}H-noradrenaline do not mix homogeneously with the total tissue store but enter a small easily releasable store.

The existence of 'functional' or 'available' pools of noradrenaline within adrenergic nerve terminals is an attractive concept since it helps to explain many aspects of drug action at the nerve terminal. This concept has been discussed by a number of authors (Iversen, 1967; Glowinski, 1970; Pellegrino de Iraldi & Suburo, 1971; Shore, 1972). Although much of the evidence for separate pools is indirect, convincing evidence for such a concept has been obtained in the case of noradrenaline newly synthesized from labelled tyrosine (Kopin, Breese, Krauss & Weise, 1968). The situation with respect to the labelling of neuronal stores with ^{3}H-noradrenaline is less clear; however the following results suggest that under certain conditions exogenous ^{3}H-noradrenaline does *not* mix homogeneously with tissue stores, and may be preferentially released during nerve stimulation.

Methods.—Male albino rabbits (2·5–3·5 kg) were killed by cervical dislocation, the vasa deferentia were removed and placed in warm modified Krebs solution of the following composition (mM): NaCl 118, KCl 4·75, $CaCl_2$ 2·54, $MgSO_4$ 1·19, $NaHCO_3$ 25, KH_2PO_4 0·93, glucose 11, tyrosine 0·025, ascorbic acid 0·1, disodium edetate 0·027. Both tissues were carefully stripped of adhering blood vessels, fat and the outer serous coat, each vas was then slit open along its longitudinal axis. The following incubation and wash cycles were then followed (Krebs solution bubbled with 95% O_2+5% CO_2 was used throughout):

(a) 20 min pre-incubation at 37° C,

(b) 45 min incubation with (−)-^{3}H-noradrenaline (10 or 100 ng/ml, specific activity 40 μCi/μg),

(c) 40 min wash period, with fresh solution at 37° C every 5 minutes,

(d) 60 min incubation with phenoxybenzamine (10 μg/ml).

The tissues were finally mounted in 3 ml organ baths (Hughes, 1972) maintained at 37° C; they were washed with fresh Krebs solution for a further 40 min before any collections were made. The total bath volume was collected for analysis 6 min after electrical field stimulation at 2 or 16 Hz for 300 pulses (1 ms rectilinear pulses of supramaximal strength). Collections were also made after a 20 min quiescent period (basal release). The tissues were removed immediately after the last collection of bathing fluid and they were then homogenized in 3+2 ml of 0·4 M perchloric acid (4° C) containing 0·1 mg/ml disodium edetate, the extracts were centrifuged at 10,000 *g* for 20 min (4° C) and the supernatants retained for analysis.

The bath fluids and tissue supernatants were chromotographed successively on Amberlite CG-120 and then on alumina columns (Hughes, Gillis & Bloom, 1969) to isolate noradrenaline from any metabolites. Aliquots of the final alumina eluates were used to determine the noradrenaline content by fluorimetric assay (O'Hanlon, Campuzano & Horvath, 1970) and the radioactivity by liquid scintillation spectrometry (Packard Model 2425).

(−)-^{3}H-Noradrenaline (Amersham Radiochemicals) was purified on alumina columns and the specific activity checked before use.

Results.—The results of 5 experiments on paired vasa deferentia are shown in Table 1. The ratio (specific activity released noradrenaline/specific activity tissue stores) was highly dependent on the initial concentration of the ^{3}H-noradrenaline used

TABLE 1. *Specific activity of ^{3}H-noradrenaline (NA) released during nerve stimulation compared to that of the tissue stores*

		Vas A			Vas B	
		Specific activity (dpm/ng NA)				
Expt.	Tissue store	2 Hz	16 Hz	Tissue store	2 Hz	16 Hz
1	274	526	353	1,915	2,260	1,850
2	224	844	339	1,500	1,524	1,678
3	262	750	771	2,821	2,708	2,216
4	188	500	350	2,162	1,498	1,490
5	246	720	400	1,715	2,249	2,425
Means	237	668	443	2,022	2,035	1,932
Mean difference from tissue store ± S.E.		431 ± 59	206 ± 90		13 ± 202	90 ± 256
Paired *t* test.		$P<0.005$	$P<0.05$		n.s.	n.s.

Paired experiments in which vas A was incubated with 10 ng/ml ^{3}H-noradrenaline and vas B with 100 ng/ml ^{3}H-noradrenaline. Each individual value is the mean of two observations, the tissues being stimulated alternately at 2 and 16 Hz at 20 min intervals. The tissue specific activity was calculated with corrections for loss of labelled and endogenous noradrenaline during stimulation. The basal release in each experiment was less than 5% of the stimulated output on a minute basis and was ignored in these calculations. (n.s. = not significant).

to label the tissue. In those tissues incubated with 10 ng/ml ^{3}H-noradrenaline, the mean ratio obtained at 2 Hz was 2·8, and at 16 Hz it was 1·9; in each case the specific activity of the released noradrenaline was significantly greater than that in the tissue. However, the ratio of the specific activities was not significantly different in those tissues incubated initially with 100 ng/ml ^{3}H-noradrenaline.

In a further series of 5 experiments both vasa deferentia were incubated with 10 ng/ml ^{3}H-noradrenaline. One tissue was stimulated 3 h after the incubation period and the other tissue 8–9 h after the incubation with ^{3}H-noradrenaline. In the former tissue the ratio of the specific activities was between 2·0–3·5, whereas it approached unity in the longer experiment. Preliminary experiments have shown that the specific activity of the released material remains significantly different from that of the tissue stores for up to 5 h after the incubation with ^{3}H-noradrenaline.

Discussion.—The results indicate that a homogeneous labelling of the tissue stores can be achieved with 100 ng/ml ^{3}H-noradrenaline, whereas at the lower concentration of 10 ng/ml the exogenous noradrenaline does not immediately mix with the total store. It was therefore possible to obtain a preferential release of ^{3}H-noradrenaline during nerve stimulation when the tissue had been incubated with 10 ng/ml ^{3}H-noradrenaline. This preferential release can be seen up to 5 h after labelling but not after 8 h, indicating that the easily 'releasable store' and the total store eventually reach an equilibrium. Further work is needed to see if the specific activities are affected by the stimulus frequency since the results show a lower specific activity of released noradrenaline at 16 Hz compared to 2 Hz. This may indicate a mobilization of different pools at different frequencies and could be related to the increase in the output of noradrenaline/pulse with increasing frequency that is seen in the rabbit vas deferens and portal vein (Hughes, 1972; Hughes & Roth, 1972).

The anatomical basis for different noradrenaline 'stores' is not clear. It is possible that 'stores' close to, or in, the neuronal membrane are preferentially labelled at low noradrenaline concentrations. These 'stores' then form an easily releasable 'pool' because of their close proximity to the release mechanism. Autoradiographic evidence in support of this hypothesis has been obtained by Budd & Salpeter (1969). Differential centrifugation studies have also indicated a high degree of labelling of light membrane components by ^{3}H-noradrenaline (Roth & Hughes, unpublished results). Preliminary work with the rabbit portal vein has yielded similar results to those described here and thus these results do not appear to be peculiar to the vas deferens.

The above results support the concept that the preferential release of the recaptured transmitter may play an important role in adrenergic transmission (Hedqvist & Stjärne, 1969), possibly greater than that of newly synthesized noradrenaline.

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