The *dextro*-isomer of noradrenaline as a false transmitter

The process of synthesis, storage and release of the transmitter acting at adrenergic nerves is not entirely specific for noradrenaline, since structurally related substances may be formed and stored in adrenergic axons and subsequently released by nerve stimulation. Such substances, which may be termed "false neurochemical transmitters", may be of value as therapeutic agents and as experimental tools. The subject of false neurotransmitters has been reviewed extensively (Crout, 1966; Kopin, 1968a, b; Carlsson, 1969; Stone, 1971).

False transmitters of noradrenergic neurons vary in affinity for the noradrenergic receptor but are generally less active than the physiological transmitter. The (+)-isomer of noradrenaline, (+)-noradrenaline, is much less potent on a number of tissues than the naturally occurring transmitter (−)-noradrenaline (Ludena, Ananenko & others, 1949; Ludena, Euler & others, 1957; Ludena & Snyder, 1964; Green & Fleming, 1968). We have compared the vasoconstrictor potency of the two isomers on the isolated perfused artery from the rabbit ear. In 12 preparations, (−)-noradrenaline was more potent than (+)-noradrenaline, the potency ratio being 669 with 95% confidence limits of 558–800. Similar potency ratios were obtained for the pressor action of the two isomers in pithed and anaesthetized rat preparations. In the pithed rat, the potency ratio was 488 with 95% confidence limits of 411–579, and in the anaesthetized rat the (−)-isomer was 650 times (511–827) more potent than the (+)-isomer. In view of the large potency difference between the isomers and the close chemical similarity, we investigated whether (+)-noradrenaline could act as a false transmitter in vascular tissue.

The accumulation of the isomers into innervated and denervated isolated arteries from the rabbit ear was studied. The arteries were denervated by removal of the left superior cervical ganglion 7 to 10 days earlier. Paired innervated and denervated arteries were excised and incubated with either (−)-[^{3}H]noradrenaline ($1{\cdot}2 \times 10^{-6}$M, 5·4 Ci/mM) or (+)-[^{14}C]noradrenaline ($2{\cdot}45 \times 10^{-4}$M, 21·2 mCi/mM) in Krebs-Henseleit solution for 60 min. The arteries were then washed for 60 min, homogenized and total radioactivity was estimated by liquid scintillation counting. The radioactivity accumulated by the innervated artery incubated in (−)-[^{3}H]noradrenaline was 6500 d/min mg protein, whereas the denervated artery accumulated only 2100 d/min per mg protein. Similarly, denervation markedly reduced the incorporation of radioactivity into arteries incubated in (+)-[^{14}C]noradrenaline, the innervated artery accumulating 6700 d/min mg protein and the denervated artery 2900 d/min per mg protein. These results indicate that both isomers are accumulated by sympathetic nerves of arteries. Previously it has been shown that (+)-noradrenaline is accumulated by rat heart (Iversen, 1963) and by rat tisues *in vivo* (Kopin & Bridgers, 1963; Maickel, Bevan & Brodie, 1963).

To show that the (+)-[^{14}C]noradrenaline could serve as a false transmitter, it was necessary to demonstrate its release by sympathetic nerve stimulation. This was achieved using a superfused artery preparation (Allen, Rand & Story, unpublished). Arteries were incubated with (−)-[^{3}H]noradrenaline, (±)-[^{14}C]noradrenaline, or (+)-[^{14}C]noradrenaline in Krebs-Henseleit solution for 60 min and then washed by perfusion and superfusion for a further 60 min. The concentrations and specific activities of the noradrenaline isomers are shown in Table 1. Spontaneous release of radioactivity was measured from three samples of the combined perfusate and superfusate collected for 30 s at 2 min intervals. Then the nerve was stimulated with 1 ms pulses at 50 Hz for 30 s, during which a further fraction was collected. The results, given in Table 1, demonstrate that the (+)-isomer of noradrenaline was released on

Table 1. *Release of radioactivity from isolated artery segments previously incubated with radio-labelled noradrenaline.* Mean d/min ± s.e.; number of artery segments in brackets.

Radio-labelled noradrenaline (NA) isomers	Molar concentration	Specific activity	Release d/min per sample	
			Spontaneous	Nerve stimulation
(−)-[^{3}H]-NA	$1{\cdot}22 \times 10^{-6}$	5400	218 ± 43 (5)	1243 ± 113 (5)
(±)-[^{14}C]-NA	$2{\cdot}73 \times 10^{-4}$	37	86 ± 9 (6)	327 ± 80 (6)
(+)-[^{14}C]-NA	$2{\cdot}45 \times 10^{-4}$	21·2	55 ± 2 (6)	151 ± 17 (6)

nerve stimulation. This same pattern of release was obtained after 5 h of washing, indicating equivalent retention of the isomers over this time period. The presence of tetrodotoxin in the perfusate (0·5 μg/ml) completely abolished the rise in radioactivity released for each of the isomers and also abolished the vasoconstrictor response to stimulation. This effect of tetrodotoxin was reversed on drug washout.

These results demonstrate that the (+)-isomer of noradrenaline can act as a false transmitter and may serve as a useful basis for further work towards the development of a therapeutic application of this substance.

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