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Release of [^{3}H]noradrenaline from vasoconstrictor nerves

SIR,—The release of noradrenaline by nerve impulses from vasoconstrictor nerves is of great cardiovascular significance. This has been examined in the perfused hind limb of the dog (Rosell, Kopin & Axelrod, 1963) and more extensively in the cat spleen (see reviews by Brown, 1965; Gillespie, 1966). However, these experiments are complicated by the presence of non-vascular smooth muscle and concurrent fluctuations of perfusion parameters during nerve stimulation.

We have investigated the noradrenaline release in the structurally simple rabbit pulmonary artery which is adrenergically innervated (Bevan & Su, 1964; Verity & Bevan, 1966). This artery was cut spirally into a strip as small as 3 × 40 mm, weighing 40 mg. It was connected under a 2 g tension to an isometric strain gauge transducer, and mounted in a 2 ml tissue bath containing Krebs solution at 37° which was constantly stirred by bubbles of oxygen 95% and carbon dioxide 5%. The intramural adrenergic nerves were stimulated by a 2 min train of square wave impulses (0·3–1 msec duration, 10 cycles/sec, near maximal voltage), using platinum wire electrodes placed on either side of the strip. The contraction following stimulation was registered on a pen recorder.

The artery strip was initially incubated in Krebs solution containing 5 μc/ml (0·486 μM) of [(±)-7-^{3}H-noradrenaline]* hydrochloride (specific activity 10·28 c/mmole) for 30 min. This medium was then flushed and plain Krebs solution introduced into the tissue bath at a constant rate (1–2 ml/min). The overflow was collected in 1 ml aliquots for assay of tritium activity by scintillation spectrometry.

Thirty min after commencement of washing out, the first period of nerve stimulation was applied, and this elicited a sharp rise in the tritium outflow and muscle contraction (Fig. 1). Both responses returned to the baseline levels within 20 min. At this interval, stimulations were repeated up to 14 times with consistent contractile responses and a constantly diminishing but significant rise in tritium outflow. The first period of stimulation brought about a disproportionately great tritium output as compared to subsequent stimulations. Thus, in Fig. 1, the first peak represented a total of 93·0 nc tritium output, whereas the second, third and fourth peaks amounted only to 19·4, 11·8 and 9·9 nc, respectively. It is possible that some [^{3}H]noradrenaline was initially present either in the extracellular space or in an easily releasable form and was expelled by the first period of stimulation. The [^{3}H]noradrenaline

* 2-Amino-1-(1,3-dihydroxyphenyl)-[1-^{3}H]ethanol.

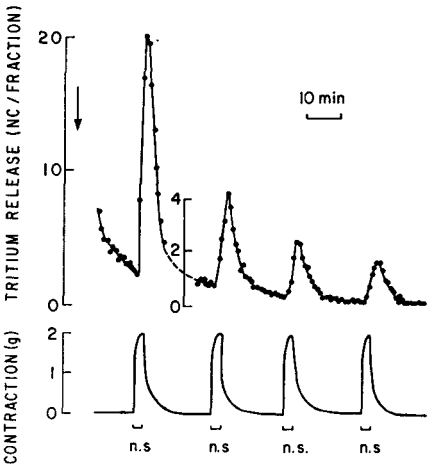

FIG. 1. Isolated rabbit pulmonary artery. After pretreatment of the artery with 5 μc/ml of [^{3}H]noradrenaline for 30 min, continuous flow (1·3 ml/min) of Krebs solution was introduced at arrow. The outflow was collected in 1 ml fractions for assay of tritium activity. Muscle tension was simultaneously recorded, and nerve stimulation applied at n.s.

subsequently released may have mixed more thoroughly with the endogenous noradrenaline and thus better represented the latter.

In several experiments, the pulmonary artery strip was incubated with [^{3}H]noradrenaline at 37° and then irrigated at 25°. When nerve stimulation was applied, delayed and diminished contraction resulted compared to that observed at 37°. The tritium outflow was also delayed and prolonged, and the total outflow per stimulation period reduced as the result of cooling.

Although the [^{3}H]noradrenaline released from the adrenergic nerves is probably in part metabolized and rebound (Axelrod, 1965), the tritium outflow under the above conditions may serve as an indicator of the neural release of endogenous noradrenaline. In view of the simplicity of the preparation and the high sensitivity of the technique, this appears to be a useful method for investigating transmitter release by vasoconstrictor nerves in particular, and the adrenergic neuroeffector transmission in general.

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