Release of [^{3}H]noradrenaline and [^{3}H]metaraminol from rabbit pulmonary artery *in vitro*

Metaraminol, a congener of noradrenaline, is taken up by adrenergic neurons from which it is released by electrical stimulation and thus may act as a "false" transmitter (Crout, Alpers & others, 1964; Shore, Busfield & Alpers, 1964). In contrast to noradrenaline, metaraminol is resistant to monoamine oxidase and catechol-*O*-methyltransferase. For this reason metaraminol has often been used as an experimental tool instead of noradrenaline for the study of adrenergic neuroeffector transmission processes (for references, see Muscholl, 1972). However, to our knowledge a direct quantitative comparison has not been made between the amounts of noradrenaline and metaraminol released from the same preparation under strictly controlled conditions. We now report that marked differences exist between the liberation of these two transmitters from an artery *in vitro*. This was the case both when electrical-field stimulation and tyramine, an indirectly acting sympathomimetic amine, were used to elicit the release.

The pulmonary artery from adult albino rabbits (1·8–2·3 kg) was excised and divided into two rings (each weighing approximately 25 mg). Each ring was suitably mounted on a plastic holder, connected to a sensitive transducer (Statham, G 10 B) and placed in an isolated tissue bath containing 2 ml physiological salt solution (PSS) at 37° (Nedergaard & Schrold, 1973) which was aerated with 5% carbon dioxide in oxygen. The tissues were incubated with either (−)-[^{3}H]noradrenaline (^{3}H-NA; 10^{-6}M; 6·41 Ci mmol^{-1}) or (±) [^{3}H]metaraminol (^{3}H-MA; 10^{-6}M; 8·23 Ci mmol^{-1}) (New England Nuclear Corporation) (both >90% pure as determined by chromatography and biological assay) for 45 min. Subsequently the bath was automatically emptied and refilled every 2 min. Thus non-specifically bound [^{3}H] amine was removed by repeated washing with PSS during a 90 min period. The rings were then subjected to electrical-field stimulation using a Grass S 4 square wave stimulator. Each period of stimulation consisted of 300 biphasic shocks at 9 V; 10 Hz and 0·3 ms followed by a 14 min rest period; the stimulation parameters used provided supramaximal and selective stimulation of adrenergic neuron fibres (Nedergaard & Schrold, 1973). In other experiments the rings were incubated with tyramine (10^{-4}M) for 10 min. The bath fluid (2 ml) containing the outflow of tritium was collected directly in counting vials and tritium was measured by liquid scintillation spectrometry (Mark II, Nuclear Chicago Corporation). At the end of each experiment the arterial content of tritium was determined after partial dissolution of the tissue by means of Protosol (Nuclear Chicago Corporation). Quenching was determined by the internal standard method using [^{3}H] water. The practical counting efficiency was 35%. The tritium outflow represents unchanged [^{3}H] amines and their [^{3}H] metabolites (Su & Bevan, 1970). However, it seems reasonable to assume that the tritium outflow directly represents the actual release of [^{3}H] amines from adrenergic neurons less their neuronal re-uptake and possible extraneuronal tissue binding. Hence, fractional outflow of tritium was corrected for tissue weight and specific radioactivity and expressed as pmol g^{-1} tissue/fraction. Thus, in the following, the forms ^{3}H-NA and ^{3}H-MA outflow refer to outflow of tritium from tissue preloaded with ^{3}H-NA and ^{3}H-MA respectively and are not corrected for properties of corresponding metabolites present in samples. Treatment-induced outflow of tritium was calculated by summation of each fraction value minus estimated passive outflow. Treatment-induced outflow is expressed either as pmol g^{-1} tissue or as per cent of total tritium content in the tissue at the onset of treatment (calculated by summation of assayed tritium outflow and tissue tritium content at the end of an experiment).

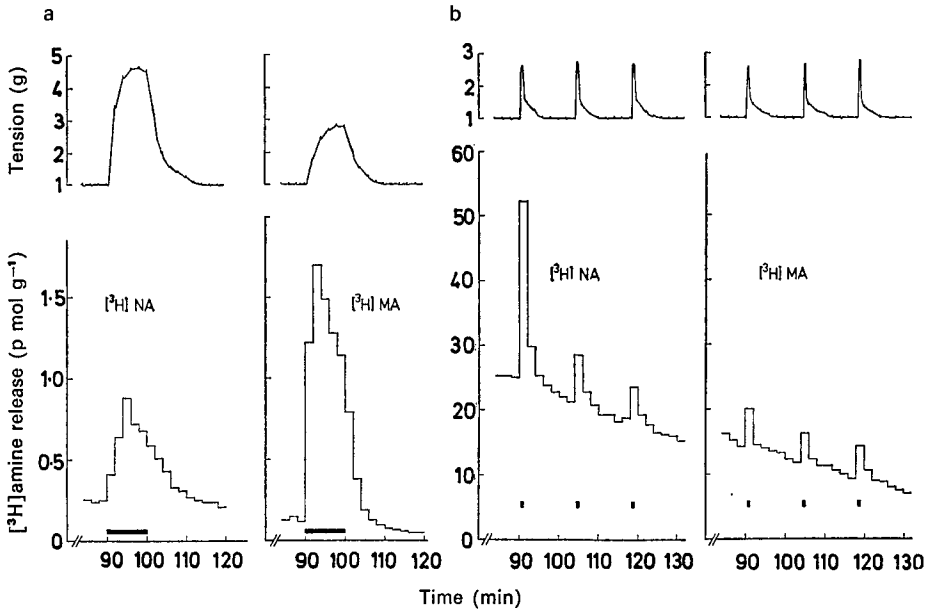

FIG. 1. Relations between tension development and [³H] amine outflow from rabbit isolated pulmonary arteries preincubated with ³H-NA and ³H-MA. *Upper half:* Tyramine (10^{-4}M) induced outflow (pmol g^{-1}) which was 302 (³H-NA) and 713 (³H-MA). *Lower half:* Field stimulation induced outflow (pmol g^{-1}) which was 32, 9 and 6 (³H-NA) and 5, 5 and 6 (³H-MA), respectively. The black bars indicate duration of treatment causing release. The concomitant contraction response is shown in the upper part of each half.

Experiments were always carried out in parallel with tissue from the same rabbit. Differences between means were determined by Student's *t*-test for paired data.

Electrical-field stimulation of the arteries caused outflow of ³H-NA and ³H-MA (Fig. 1). Repeated stimulation caused the outflow of ³H-NA to decline markedly after the first period of stimulation while the ³H-MA outflow remained almost the same (Table 1). This was also the case when 1000 electrical pulses were used instead of 300, although the absolute amount of tritium outflow was enhanced. It is unlikely that the initial stimulus-induced high outflow of ³H-NA is an artifact, say due to release of

Table 1. *Outflow of ³H-NA and ³H-MA from rabbit isolated pulmonary arteries caused by electrical-field stimulation and tyramine.* Arteries were incubated with ³H-NA or ³H-MA (both 10^{-6}M) and then washed with PSS for 90 min. Subsequently the arteries were either stimulated for three consecutive periods (I, II and III) at 14 min intervals or incubated with tyramine (10^{-4}M). [³H] outflow is expressed as per cent of tissue [³H] content at the onset of treatment (see text). Values represent mean ± s.e.

Treatment	No	Number of observations	[³H]outflow (%) ³H-NA	³H-MA
Field stimulation	I	5	1·58 ± 0·28 *	0·39 ± 0·10
	II	5	0·64 ± 0·09 *	0·34 ± 0·06
300 pulses	III	5	0·57 ± 0·08	0·48 ± 1·11
Tyramine (10^{-4}M)	I	6	13·7 ± 0·8 **	46·0 ± 1·9

* Differs from ³H-MA value ($P < 0.05$)
** Differs from ³H-MA value ($P < 0.01$)

extraneuronal non-specifically bound ^{3}H-NA, since the same phenomenon was not observed with ^{3}H-MA. The decline in outflow of ^{3}H-NA cannot be due to lack of this amine in the artery since 91% of ^{3}H-NA remained in the vessel after the first period of stimulation. The difference between outflow of ^{3}H-NA and ^{3}H-MA is probably not due to the use of stereoisomers with different optic activity ((−)-isomer of ^{3}H-NA and (±)-isomer of ^{3}H-MA), since the outflow of (−)- and (±)-isomers of ^{3}H-NA was the same (unpublished). We confirmed the observation (Su & Bevan, 1970) that there is a clear dissociation between the stimulus-induced ^{3}H-NA outflow and contraction height (Fig. 1). While the former decreased, the latter remained constant with repeated stimulation. This was not the case with ^{3}H-MA. These results indicate that the ratio tritium outflow/endogenous non-radioactive noradrenaline is variable for tissue preloaded with ^{3}H-NA but constant for that preincubated with ^{3}H-MA.

Tyramine (10^{-4}M) released ^{3}H-NA and ^{3}H-MA (Fig. 1). Tyramine-induced outflow of these [^{3}H] amines was much higher (up to 138 times) than that seen with field stimulation (Table 1). In contrast to the latter treatment (first period of stimulation) tyramine caused a higher (about 3 times) outflow of ^{3}H-MA than of ^{3}H-NA. This may possibly in part be due to a difference in intraneuronal disposition of these [^{3}H] amines. The tyramine-induced outflow of [^{3}H] amines and contraction height showed an inverse relation (Fig. 1).

The present results indicate that the differences in release of ^{3}H-NA and ^{3}H-MA must be taken into account when metaraminol is used instead of noradrenaline as an experimental tool.

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Myocardial depressant action of ethyl acetate

Ethyl acetate is one of the most frequently used organic solvents (see, for example, Deichmann & Gerarde, 1969), but in spite of its extensive use and its not infrequent abuse (Smart, Fejer & Alexander, 1972), information on its toxicological properties is scanty (Beintker, 1928; Smyth & Smyth, 1928; Blina, 1933; Mancini, Noferi & others, 1943).

We have investigated the effect of ethyl acetate on myocardial contractility in guinea-pig isolated ventricular strips to compare its toxicity with that of ethanol.

Guinea-pigs of either sex, of about 500 g, were killed by cervical dislocation. The hearts were removed immediately and the right ventricle excised. The ventricular strip (1 × 10 mm) was suspended in a bath containing oxygenated Ringer-Locke solution (32°; pH 7·3) through which a gas mixture of 5% CO_2 in oxygen was bubbled