Effects of induced sodium gradients on the transport of metaraminol

Noradrenaline and metaraminol are accumulated by tissues by a process which exhibits saturation kinetics (Iversen, 1963), is Na^+- and K^+-dependent (Iversen & Kravitz, 1966; Gillis & Paton, 1967; Bogdanski, Tissari, & Brodie, 1968; Bogdanski & Brodie, 1969), requires energy (Paton, 1968) and is ouabain-sensitive (Bogdanski & Brodie, 1969). The requirements for amine transport are thus similar to those of the Na^+- and K^+-activated ATPase. Bogdanski & Brodie (1969) have postulated that noradrenaline transport occurs as the result of the operation of a process similar to those proposed by Crane (1965) and Kipnis & Parrish (1965) to account for the transport of sugars and amino-acids by various tissues. According to this model, noradrenaline transport results from an interaction with a membrane carrier the affinity of which for the amine is Na^+-dependent. The carrier transports amine and Na^+ intracellularly where the affinity of the carrier for the amine falls thus releasing amine. Inward noradrenaline transport continues as long as the inward Na^+ gradient is maintained by the extrusion of Na^+ from the cell by the Na^+ pump.

An analysis of this model leads to the predictions that, by driving a net inward Na^+ flux, an electrochemical gradient of Na^+ will drive a net inward flux of amine and that this will continue in the absence of Na^+ pumping as long as an inward Na^+ gradient is maintained (Stein, 1967). The applicability of this prediction to metaraminol transport has been examined by determing the ability of an induced inward Na^+-gradient to produce transport of metaraminol when the Na^+- and K^+-activated membrane ATPase is inhibited.

Male New Zealand white rabbits were killed by a blow on the head after which their hearts were excised rapidly and slices of left ventricle prepared as described previously (Gillis & Paton, 1967; Paton, 1968). Slices were incubated in medium at 37° and gassed with carbon dioxide 5% in oxygen. During a preincubation of 30–60 min all slices were in low Na^+ medium of the following composition (mmol/litre): $NaHCO_3$ 22, NaH_2PO_4 1·2, $MgCl_2$ 1·2, $CaCl_2$ 2·5, Na_2 EDTA 0·03, sucrose 232, KCl 5·0, (+)-glucose 10. Where indicated KCl and (+)-glucose were replaced iso-osmotically with sucrose. Following preincubation slices were placed in either low Na^+ medium or a normal Na^+ medium in which sucrose was replaced by NaCl 116 mmol/litre, [^{3}H]-metaraminol added to achieve a final concentration of 2×10^{-8}M of the free base and the incubation continued for a further 6 or 30 min. During preincubation and incubation with [^{3}H] metaraminol the Na^+ pump was inhibited by either removal of K^+ or 10^{-5}M ouabain or low temperature (4°) or metabolic inhibition; full details are given in Table 1. The slices were then digested using NCS Solubiliser (Amersham Searle) and the content of [^{3}H]amine measured as described previously (Paton, 1968). Retention of [^{3}H]metaraminol was expressed as a ratio (R) calculated by dividing the [^{3}H]disintegrations/min g^{-1} of slices by [^{3}H] disintegrations/min ml^{-1} of medium. Chromatographically pure (±)-metaraminol-7-[^{3}H]-hydrochloride with a specific activity of 6·5 Ci/mmol was obtained from the New England Nuclear Corporation.

The results obtained are shown in Table 1. Slices were preincubated in low Na^+ medium to prevent Na^+ enrichment which would have otherwise occurred since the Na^+ pump was inhibited. Uptake of [^{3}H] metaraminol was measured after 6 min as well as 30 min because Eddy, Mulcahy & Thomson (1967) have shown that under similar conditions the maximal uptake of glycine by ascites-tumour cells occurred within 10 min, the subsequent decline in uptake of glycine apparently being produced by a rise in intracellular Na^+ with a consequent reduction in the inward Na^+ gradient. It can be seen that when the Na^+ pump was not inhibited, the effects of low Na^+

Table 1. *Influence of Na^+ gradient on the uptake of* [^{3}H]*metaraminol by rabbit heart slices*

Group	Preincubation	Incubation with [^{3}H] metaraminol	R value (mean ± s.e.) 6 min	30 min
A	Low Na^+ medium (30 min)	Low Na^+ medium	1·86 ± 0·27 (12)	3·52 ± 0·32 (18)
		Normal Na^+ medium	2·73 ± 0·26 (12)	8·15 ± 0·59 (18)
B	Low Na^+ medium plus 10^{-5}M ouabain (30 min)	Low Na^+ medium plus 10^{-5}M ouabain	1·20 ± 0·13 (6)	2·47 ± 0·10 (7)
		Normal Na^+ medium plus 10^{-5}M ouabain	1·13 ± 0·13 (6)	1·75 ± 0·07 (9)
C	Low Na^+, K^+ free medium (60 min)	Low Na^+, K^+ free medium	1·58 ± 0·10 (8)	3·49 ± 0·21 (8)
		Normal Na, K^+ free medium	1·20 ± 0·16 (8)	1·97 ± 0·13 (8)
D	Low Na^+ medium at 4°	Low Na^+ medium at 4°	0·68 ± 0·04 (6)	1·18 ± 0·10 (6)
		Normal Na^+ medium at 4°	0·68 ± 0·16 (6)	0·96 ± 0·07 (6)
E	Low Na^+, glucose free medium (60 min) with 10^{-3}M dinitrophenol (40 min) and 10^{-3}M iodoacetic acid (20 min).	Low Na^+ glucose free	1·09 ± 0·04 (10)	1·79 ± 0·07 (10)
		Normal Na^+ glucose free	0·96 ± 0·08 (10)	1·55 ± 0·07 (10)

Number in brackets is no. of slices in each group.

medium on uptake were rapidly reversible, an increase in Na^+ gradient resulting in an immediate increased uptake of [^{3}H] metaraminol (Group A). However when the Na^+ pump was inhibited by either 10^{-5}M ouabain (Group B), absence of external K^+ (Group C), low temperature (4° C) (Group D) or metabolic inhibition (Group E), an increase in the Na^+ gradient did not increase the uptake of [^{3}H]metaraminol after either 6 or 30 min incubation. An inward directed Na^+ gradient similarly failed to increase the transport of [^{3}H]noradrenaline into synaptosomes pretreated with ouabain (Tissari, Schönhöfer & others, 1969) or metabolic inhibitors (White & Keen, 1970).

An analysis of the proposed model for noradrenaline transport also leads to the prediction that an induced outward Na^+ gradient should produce transport of noradrenaline out of the cell against the concentration gradient as has been reported for sugar transport by intestine (Crane, 1965). This phenomenon could not however be demonstrated for metaraminol transport using ventricular slices obtained from animals pretreated with reserpine, 2 mg/kg intravenously 24 h previously. Slices were first incubated in normal Na^+ medium containing [^{3}H]metaraminol for 3 min and then transferred to a Na^+-free medium containing [^{3}H]metaraminol for an additional 10 or 20 min. The R values obtained were 1·15 ± 0·11 (12) at 3 min, 1·62 ± 0·06 (9) at 13 min and 1·82 ± 0·04 (10) at 23 min.

The findings outlined here together with those recently reported by Tissari & others, (1969) and White & Keen (1970) have shown that an inwardly directed Na^+-gradient could not serve as the only driving force for the transport of noradrenaline and metaraminol. Similarly an outwardly directed Na^+-gradient did not reverse the direction of amine transport. It thus seems likely that the mechanism for noradrenaline transport postulated by Bogdanski & Brodie (1969) requires modification. The activity of the Na^+- and K^+-activated ATPase appears essential for noradrenaline and metaraminol transport and may be required to provide energy for transport or to phosphorylate the amine carrier.

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Reduced variation of [^{3}H]noradrenaline uptake into rat submaxillary glands by atropine pretreatment

The submaxillary gland of the rat is useful for the study of peripheral adrenergic mechanism. It has a rich adrenergic innervation with a mean noradrenaline content of about 1·2 μg/g tissue, is paired so that it is always possible to get a matched control, and is easily accessible for different mechanical manipulations. The adrenergic nerves of the gland, with cell bodies in the superior cervical ganglion, and the preganglionic fibres in the cervical sympathetic trunk, are easily reached from the neck.

In the studies made in this laboratory using the preparation a serious problem was encountered in the great variation between different animals in the uptake of labelled noradrenaline (^{3}H-NA) or its analogues. A possible explanation for this could be that the secretory activity of the submaxillary gland and thus the blood flow through the organ varied widely between and within animals. The following experiment was made to see if a uniform increase or decrease of the secretory activity of the submaxillary gland could reduce the differences in uptake of labelled amines.

Male Sprague-Dawley rats, 170–270g, kept at 31°, received 1 μg/kg ^{3}H-NA (specific activity 8·45 Ci/mmol, NEN Chemicals) in a tail vein. One group of 8 rats was pretreated with atropine, 1 mg/kg intraperitoneally 30 min before the ^{3}H-NA injection. In another group, 8 rats were forced to chew dry wheat starch 5 min before and 5 min after the ^{3}H-NA injection. A third group, also of 8 rats, was not pretreated. Three h after the ^{3}H-NA administration the rats were killed. The hearts and the submaxillary + sublingual glands were immediately taken out, weighed and homogenized in ice-cold 0·4 N perchloric acid. The salivary glands from both sides were analysed together. Noradrenaline was separated on cation exchange columns and the tritium contents of the eluates were measured by liquid scintillation counting (Carlsson & Waldeck, 1963; Stitzel & Lundborg, 1966).

Three h after the ^{3}H-NA injection a mean 1·58 ng/g (variance 0·297) ^{3}H-NA was found in the submaxillary + sublingual glands. Neither after pretreatment with atropine (1·41 variance 0·041) nor with dry wheat starch (1·50 variance 0·304) was this