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A possible augmentation of the extraneuronal adrenaline uptake caused by inhibition of the neuronal component

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It is well known that when a high concentration of a catecholamine such as adrenaline is applied to adrenergically-innervated preparations, the amine is rapidly taken up into both the neuronal ($Uptake_1$) and extraneuronal ($Uptake_2$) sites (Iversen, 1965; Gillespie & Hamilton, 1966; Draskóczy & Trendelenburg, 1970). The manner of the amine uptake into the extraneuronal site differs from that into the neuronal site in many respects, such as energy dependency and sensitivity to various uptake inhibitors, but the precise mechanism involved remains obscure. In the present experiments, an attempt has been made to determine whether the amount of adrenaline taken up into the extraneuronal compartment is altered when transport across the axonal membrane is inhibited.

There is clear-cut evidence (Hertting, 1964; Callingham & Burgen, 1966; Ross & Renyi, 1966) that isoprenaline can be taken up into the extraneuronal, but not into the neuronal site. Thus, the transport of this amine under the conditions described above has also been examined in comparison with adrenaline, which is capable of being taken up into both sites (Iversen, 1965; Draskóczy & Trendelenburg, 1970).

The vas deferens isolated from a male guinea-pig was suspended in a bath (1 ml) containing a tris buffered saline solution pH 7·4 described by Paton (1973) and bubbled with O_2. Throughout the experiment, the bathing medium was maintained at approx. 37°. After equilibration for 1 h, the preparation was loaded with (±)-adrenaline or (±)-isoprenaline (3×10^{-5} M) for 15 min, rapidly washed three times with amine-free medium, and then kept for 20 min in this medium. This washout period was used since previous results (Bönisch, Uhlig & Trendelenburg, 1972; Lindmar & Löffelholz, 1972; Katsuragi & Suzuki, 1976) have shown that the spontaneous release of the total extraneuronal amine occurs long before that of the neuronal amine. The amounts of the catecholamine released into the final washout medium were fluorimetrically determined by the trihydroxyindole method at pH 3·5 and pH 7·0 as described by Katsuragi & Suzuki (1977). The values of the amines, determined at pH 3·5 to avoid contamination from the endogenous noradrenaline, are shown in Figs 1 and 2. Also, the mandelic acid metabolites of adrenaline, produced by monoamine oxidase, did not affect the adrenaline fluorescence, while any endogenous metanephrine, produced by catechol-*O*-methyltransferase, was discriminated from adrenaline by the method used in a previous study (Katsuragi & Suzuki, 1977). Adrenaline and isoprenaline were measured at 335/510 and 330/500 nm, respectively. It has been assumed that the amount of amine found in both fluids after 20 min is a measure of the amount of uptake into the extraneuronal tissue of the vas deferens.

As shown in Fig. 1, surgical denervation (1 week previously; Birmingham, 1970) or addition 10 min before loading with amine or cocaine (10^{-5} M), desipramine ($3·8 \times 10^{-5}$ M) or ouabain (10^{-4} M), which inhibit neuronal amine uptake, greatly enhanced the spontane-

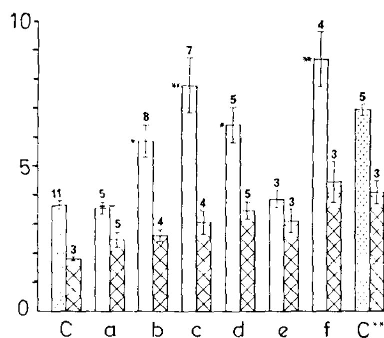

FIG. 1. Effects of blockade of neuronal amine uptake on the efflux of adrenaline output from extraneuronal-compartments in guinea-pig vas deferens. Drugs, including clonidine ($1·9 \times 10^{-4}$ M), but not reserpine, were added to the bath 10 min before the loading of adrenaline. Values of adrenaline uptake are shown by amounts (n mol g^{-1} wet weight of tissue) (ordinate) of spontaneous release of the amine in the bath throughout the washout period. Vertical bars: mean ±s.e. Number in parentheses represent the number of experiments. Control***: loading with 9×10^{-5} M adrenaline. ** Differ from control, $P < 0·01$; * differ from control, $P < 0·05$. C: Control; a: reserpine; b: denervation; c: cocaine (10^{-5} M); d: DMI ($3·8 \times 10^{-5}$ M); e; ouabain (10^{-5} M); f: ouabain (10^{-4} M). Cross-hatched columns show pretreatment with clonidine. Stippled columns show control uptake with clonidine.

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ous outflow of adrenaline from the vas deferens. The increased amine outflow following these drugs was greatly reduced by the addition of clonidine ($1{\cdot}9 \times 10^{-4}$ M), an $Uptake_2$ inhibitor (Salt, 1972). Clonidine, unlike metanephrine (Iversen, 1965) or 3-methoxyisoprenaline (Mireylees & Foster, 1973), is suitable as the inhibitor since it does not interfere with catecholamine fluorescence. Pretreatment of the guinea-pig with reserpine (1st day 2 mg, 2nd and 3rd days 1 mg kg^{-1}, i.p. for 3 days), or ouabain (10^{-5} M) added to the bath did not alter the efflux of amine into the bathing fluid. The values of the enhanced amine output were similar to the value obtained when the loading concentration of adrenaline was raised 3-fold. The amount of the amine in the bathing medium at the end of the adrenaline loading period was scarcely altered by inhibition of neuronal uptake.

When isoprenaline was used to load the tissue, pyrogallol (10^{-4} M) and pargyline (10^{-4} M) were added to the bath 10 min before to inhibit catechol-*O*-methyltransferase and monoamine oxidase. The control efflux of isoprenaline, in contrast to that of adrenaline, was unaffected by inhibition of neuronal uptake as shown in Fig. 2. However, the efflux of isoprenaline was significantly reduced by the preaddition of clonidine ($1{\cdot}9 \times 10^{-4}$ M).

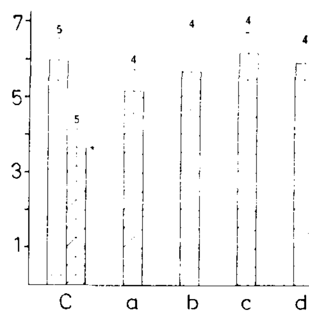

FIG. 2. Effects of blockade of neuronal amine uptake on the efflux of isoprenaline from extraneuronal compartments in the guinea-pig vas deferens. Drugs were added into the bath 10 min before the isoprenaline loading. Values of isoprenaline output are shown by amounts (n mol g^{-1} net weight of tissue) (ordinate) of spontaneous release of the amine in the bath throughout the washout period. Vertical bars: mean ± s.e. Number in parentheses represents the number of experiments. Cross-hatched column, clonidine ($1{\cdot}9 \times 10^{-4}$ M)-pretreatment as described in Fig. 1. *Differ from control (left stippled column), $P < 0{\cdot}001$ in paired comparison. C: Control; a, denervation; b, cocaine (10^{-5} M); c, DMI ($3{\cdot}8 \times 10^{-5}$ M); d, ouabain (10^{-4} M).

The values for the output of adrenaline and isoprenaline found in the present experiments may be regarded as proportional to the amounts of amines taken up mainly into the extraneuronal compartment of the vas deferens since the various additions of drugs were carried out before the amine loading and the measurements made during the washout period. Thus, these findings provide evidence that the extraneuronal uptake of adrenaline is significantly increased by chronic denervation and the inhibition of neuronal uptake by drugs. This would agree with the recent evidence (Su, Bevan & others, 1977) that the extraneuronal uptake of [^{3}H]noradrenaline in the carotid artery of the lamb foetus functions preferentially in the early term of gestation, and thereafter, decreases gradually with the development of sympathetic nerve function. The possibility that denervation and the drugs used in these experiments change the amine permeation of the postsynaptic cell membrane to the extraneuronal compartment (mainly the smooth muscle) can be ruled out because they had no effect on the amount of isoprenaline in the bathing fluid, and adrenaline and isoprenaline appear to be taken up into the same clonidine-sensitive extraneuonal site.

Because adrenaline, unlike to isoprenaline, has a high affinity (Ross & Renyi, 1966; Draskóczy & Trendelenburg, 1970) for the neuronal membrane, blockade of the active transport of adrenaline into the axoplasm causes the amine to flow into the post-synaptic extraneuronal compartment, which may exist in the intracellular smooth muscle (Gershon, Hagopian & Nunez, 1974; Nicholas, Strum & others, 1974), through the mechanism of facilitated diffusion (Gillespie, 1976). In this compartment, the catecholamine would be loosely retained as a result of the activity of Na^+–K^+ activated ATPase (Katsuragi & Suzuki, 1976, 1977; Katsuragi, Fukushi & Suzuki, 1978).

In conclusion, it is suggested that the increase in the amount of adrenaline, found in the bathing fluid after the addition of cocaine, desipramine or 10^{-4} M ouabain to the guinea-pig vas deferens or following chronic denervation of the organ, is due to an increase in extraneuronal uptake of the amine as a result of the block of neuronal uptake. Block of neuronal uptake of adrenaline removes the competition from this source for the amine in the extracellular fluid thus making more available to be taken up into the extraneuronal sites, from whence it is readily released again when the bathing fluid is replaced with amine-free solution.

May 5, 1978

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Effects of 5,7-dihydroxytryptamine and *p*-chlorophenylalanine on temperature regulation in rats

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Some recent studies suggest that there is input from the peripheral thermoreceptors to synaptic relays in the serotoninergic cell bodies in the midbrain and thence to the hypothalamic controller via the serotoninergic pathways. In rats, intraventricular injection of 5-hydroxytryptamine (5-HT) reduces body temperature (Feldberg & Lotti, 1967; Myers & Yaksh, 1968). Furthermore serotoninergic neurons within the midbrain raphe nuclei receive an input arising from thermoreceptors in both the skin (Dickenson, 1976; Jahns, 1976) and the midbrain area (Cronin & Baker, 1976; Hori & Harada, 1976). In addition, electrical stimulation of raphe nuclei in cats also influenced the unit activity of hypothalamic neurons which were responsive to hypothalamic temperature (Eisenman, 1974). However, several studies that have examined body temperature after brain 5-HT depletions have produced conflicting results. For example, in rats, in which brain 5-HT had been depleted by systemic administration of *p*-chlorophenylalanine (PCPA), the rise in rectal temperature on acute heat stress (38°) was reduced (Williams & Moberg, 1975). Both rats and monkeys treated with intrahypothalamic injection of 5,6-dihydroxytryptamine, a specific 5-HT depletor, showed acute increases in body temperature and thereafter were unable to maintain a normal body temperature when exposed to warm or cold environments (Myers, 1975; Waller, Myers & Martin, 1976). The destruction of brain 5-HT neurons by pretreatment with intraventricular 5,7-dihydroxytryptamine (5,7-DHT), which lowered the brain 5-HT concentration, did not disrupt the thermal balance in rabbits (Lin & Stitt, 1976; Lin, 1977; Lin, Pang & others, 1978). Moreover, little is yet known about the effects of 5,7-DHT and PCPA treatment on thermoregulatory responses of rats to different ambient temperatures. Thus, in the present study, we have used both 5,7-DHT and PCPA to decrease the brain 5-HT concentration. The alterations in the thermoregulatory responses (including body temperature, metabolic heat production and vasomotor activity) to different ambient temperatures were assessed in unanesthetized animals.

These experiments were on male Sprague-Dawley rats, initially 250–300 g, which before use were housed individually in wire-mesh cages in a room of 25 $\pm 0{\cdot}5°$ with a 12 h light-dark cycle. They were given free access to tap water and granular chicken feed. Three groups of animals were studied: 1) 0·9% NaCl (saline) vehicle-injected control rats, (2) rats that had received an intraventricular injection of the neurotoxin 5,7-DHT (100 μg in 5 μl, 3rd cerebral ventricle), and (3) rats that had received intraperitoneal injection of the tryptophan hydroxylase inhibitor PCPA (300 mg kg^{-1}). All drugs were dissolved in pyrogen-free sterile saline and were prepared in pyrogen-free glassware previously baked at 180° for 4 h. The drugs were 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT, Regis) and *p*-chlorophenylalanine methyl ester HCl (PCPA, Pfizer). For the intraventricular injection, the cannulae guide tubes were implanted in the animals under general anaesthesia (sodium pentobarbitone, 6 mg per 100 g, i.p.) according to (Lin 1977, 1978). The cannulae were located in the third cerebral ventricle. An injection cannula was lowered through the guide tube. Intraventricular location of the cannula was confirmed by allowing the 5 μl

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