other than tyrosine hydroxylase. Our results also show that disulfiram can inhibit noradrenaline and adrenaline synthesis in the adrenal glands as it does in the heart and brain.

In another experiment, rats were treated with 400 mg/kg of disulfiram intraperitoneally—the dose used by Goldstein & Nakajima to block brain and heart catecholamine turnover in cold-exposed rats. None of the treated rats survived the 3 hr of forced exercise. Goldstein & Nakajima mentioned that disulfiram-treated animals were sensitive to cold exposure. Our experiment shows that they are also very sensitive to exercise. Doses up to 1 g/kg of disulfiram were not lethal for up to three days in rats that were not exercised.

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References

Goldstein, M., Anagnoste, B. & Lauber, E. (1964). *Life Sci.*, **3**, 763–767.
Goldstein, M., Lauber, E. & McKereghan, M. R. (1965). *J. biol. Chem.*, **240**, 2066–2072.
Goldstein, M. & Nakajima, K. (1966). *Life Sci.*, **5**, 175–179.
Gordon, R., Spector, S., Sjoerdsma, A. & Udenfriend, S. (1966). *J. Pharmac. exp. Ther.*, **153**, 440–447.
Musacchio, J. M., Kopin, I. J. & Snyder, S. (1964). *Life Sci.*, **3**, 769–775.
Shore, P. A. & Olin, J. S. (1958). *J. Pharmac. exp. Ther.*, **122**, 295–300.

Increased uptake of noradrenaline in the rat submaxillary gland during sympathetic nerve stimulation

SIR,—Uptake of noradrenaline in various organs innervated by adrenergic nerves has been studied extensively by a number of authors. However, it is not known whether this uptake mechanism is a stationary process or if there are factors which regulate the uptake of the neurotransmitter according to physiological state of the adrenergic neurons. This kind of information is important from the viewpoint that the reuptake of noradrenaline is regarded as the major pathway of the nerve impulse-released amine (Hertting & Axelrod, 1961; Rosell, Kopin & Axelrod, 1963; Gillespie & Kirpekar, 1965) and that the need for replacement of noradrenaline in the nerve endings as well as the amount of noradrenaline released would be increased when nerve activity increases. Gillis (1963) has shown that stimulation of the cardio-accelerator nerves caused an increased retention of noradrenaline by cat atria but not by ventricles while Blakeley & Brown (1964) observed a decreased uptake by the cat perfused spleen during nerve stimulation. The present paper shows that the uptake mechanism is enhanced on increasing the sympathetic activity in the submaxillary gland of rats.

Long Evans rats of either sex, weighing 250–300 g, were anaesthetized with intravenous injection of chloralose, 60 mg/kg. The rats were prepared for the stimulation of the sympathetic nerve to submaxillary gland according to the procedure described by Sedvall & Kopin (1967). The cervical sympathetic trunk and vagus nerve of one side were ligated and freed from the carotid artery and cut at the level of the clavicle. The vagus nerve was freed from the superior cervical ganglion and cut to interrupt afferent vagal impulses. The sympathetic nerve trunk was stimulated intermittently (10 sec each min) with rectangular pulses (5–7 V, 5 msec duration) at a frequency of 20/sec. The effectiveness of electrical stimulation was confirmed by salivation and wide opening of the eye

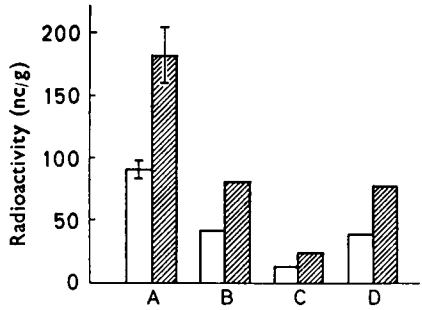

FIG. 1. [3H]Noradrenaline, [3H]normetanephrine and acid metabolites in the control (open columns) and stimulated (hatched columns) submaxillary glands of rats. A. Total. B. Noradrenaline. C. Normetanephrine. D. Acids.

of the stimulated side. The contralateral submaxillary glands served as control. (±)[^{3}H]noradrenaline (20 μc in 2 ml, 1400 mc/mM) was slowly infused into left femoral vein during a 30 min-period. At the end of infusion, electrical stimulation was interrupted. After resting for another 30 min the animals were killed and both submaxillary glands were rapidly excised and washed in cold Krebs solution. The glands were homogenized in 5 ml of chilled 0·4 N perchloric acid. [^{3}H]noradrenaline and its metabolites were isolated as described by Whitby, Axelrod & Weil-Malherbe (1961) and counted in a liquid scintillation spectrometer.

Table 1 shows that the intermittent stimulation of the cervical sympathetic trunk caused an increase of the radioactivity in the ipsolateral submaxillary glands by about 100% (23–163%). Analysis of the metabolites indicates that there was proportional increase of [^{3}H]noradrenaline as well as [^{3}H]normetanephrine and acid metabolites (Fig. 1). Desmethylimipramine (10 mg/kg; i.p.) abolished this increased uptake of [^{3}H]noradrenaline while the salivation evoked by the sympathetic stimulation as well as the resting uptake in the control submaxillary glands was not changed. This observation indicates that the increase of [^{3}H]noradrenaline uptake in the glands by nerve stimulation is not the result consequent to changes in blood flow or salivary secretion induced by sympathetic stimulation. Since both sides of submaxillary glands were exposed to the same concentration of [^{3}H]noradrenaline, it may be concluded that nerve impulses enhance the uptake mechanism of the nerve endings. The extent of enhancement may be actually more than we observed because a 30 min period of intermittent stimulation decreased respectively about 15% of endogenous noradrenaline and 30% of [^{3}H]noradrenaline in the submaxillary glands.

TABLE 1. EFFECT OF SYMPATHETIC NERVE STIMULATION ON THE UPTAKE [^{3}H]NORADRENALINE IN THE RAT SUBMAXILLARY GLAND

No. of expt.	Radioactivity in the submaxillary gland (mμc/g)		S−C	(S−C)/C
	Control (C)	Stimulated (S)		
1	65·8	80·6	14·8	0·23
2	116·6	184·9	68·3	0·59
3	90·3	220·5	130·2	1·36
4	80·2	211·3	131·1	1·63
5	90·6	234·4	143·8	1·58
6	97·4	160·9	63·5	0·65
Mean	90·2	182·1	91·9	1·01
Standard error	7·03	23·1	20·6	0·23

Whether this increased uptake caused by nerve activity is mediated by a mechanism similar to that of resting uptake is not known. The different effects of desmethylimipramine are suggestive of different mechanisms involved. A similar inference has been made by Geffen (1965) and by Boullin, Costa & Brodie (1967), although in their experiments desmethylimipramine and cocaine, in contrast to phenoxybenzamine, appear not to inhibit the nerve impulse-induced uptake. It remains to be elucidated whether the mechanism of nerve impulse-induced uptake of infused noradrenaline shown in the present experiment is similar to that shown by Geffen (1965) and Boullin & others (1967) for the uptake of nerve impulse-released amine.

Several factors which accompany nerve activity might account for the increased uptake. These are: increased permeability or active transport of noradrenaline in the nerve endings; increased noradrenaline concentration around the nerve endings, which consequently increases the extent of exchange of the amine; decrease of the store of available noradrenaline on repetitive stimulation and so increasing uptake, and an increased normetanephrine concentration due to increased release of noradrenaline. Since normetanephrine is known to enhance the uptake of noradrenaline in submaxillary glands of rats (Iversen, Fischer & Axelrod, 1966) and part of the impulse-released noradrenaline is metabolized to normetanephrine (Hertting & Axelrod, 1961), it follows that normetanephrine formed at sympathetic nerve terminals may be the mediator of the increased uptake. It is interesting that at the adrenergic nerve endings there exists such a feed-back system for the conservation of noradrenaline stores and for the disposition of the released neurotransmitter. We have also found that in the isolated vas deferens preparation of rats, transmural stimulation also caused an increase of the uptake of [^{3}H]noradrenaline. The reason why in the cat perfused spleen, sympathetic stimulation caused an impairment of uptake mechanism (Blakeley & Brown, 1964) is not known. It is interesting to note that the submaxillary gland and vas deferens are the only organs in which Iversen & others (1966) have found increased uptake of [^{3}H]noradrenaline by normetanephrine in rats.

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References

Blakeley, A. G. H. & Brown, G. L. (1964). *J. Physiol. Lond.*, **172**, pp. 19–21.
Boullin, D. J., Costa, E. & Brodie, B. B. (1967). *J. Pharmac. exp. Ther.*, **157**, 125–134.
Geffen, L. B. (1965). *J. Physiol. Lond.*, **181**, pp. 69–70.
Hertting, G. & Axelrod, J. (1961). *Nature, Lond.*, **192**, 172–173.
Gillespie, J. S. & Kirpekar, S. M. (1965). *J. Physiol. Lond.*, **176**, 205–227.
Gillis, C. N. (1963). *Biochem. Pharmac.*, **12**, 593–595.
Iversen, L. L., Fischer, J. E. & Axelrod, J. (1966). *J. Pharmac. exp. Ther.*, **154**, 56–63.
Rosell, S., Kopin, I. J. & Axelrod, J. (1963). *Am. J. Physiol.*, **205**, 317–321.
Sedvall, G. C. & Kopin, I. J. (1967). *Life Sci.*, **6**, 45–51.
Whitby, L. G., Axelrod, J. & Weil-Malherbe, H. (1961). *J. Pharmac. exp. Ther.*, **132**, 193–201.