It is of immediate interest that modification of the piperidine ring in the region of the basic nitrogen atom leads to the production of drugs in which antitussive actions are dissociated from some other morphine-like properties.

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Uptake of [^{3}H]noradrenaline in the rat heart during increased sympathetic nervous activity associated with cold

SIR,—It is known that tissue with adrenergic innervation including the heart can take up noradrenaline from the blood or from the surrounding fluid. After administration, [^{3}H]noradrenaline crosses the neuronal membrane of the sympathetic nerve endings into the cytoplasm. It is then taken up and retained within dense core vesicles where it gradually equilibrates with endogenous noradrenaline stores. [^{3}H]Noradrenaline is released in response to sympathetic nerve stimulation, Hertting & Axelrod (1962), and is inactivated enzymatically or by re-uptake and binding in the sympathetic neuron. The physiological importance of noradrenaline re-uptake may consist not only in termination of its influence on the receptor sites of the effector organ, but also in the conservation of the sympathetic transmitter. Gillis, Schneider & others (1965) have shown that increased sympathetic nervous activity results in an increased retention of [^{3}H]noradrenaline. They found, after labelling the endogenous stores of catecholamines 3 hr before the experiment, that continuous stimulation of the sympathetic nerves of the isolated atria for 50 min (at a rate of 10 shocks/sec) caused an increase in the specific activity of noradrenaline recovered from the atria. Similarly Chang & Chiueh (1968) observed that the intermittent stimulation of the cervical sympathetic trunk caused an increase of radioactivity in submaxillary glands. Less information is available about this mechanism *in vivo*. The present study was undertaken to determine whether or not there is an increased retention of [^{3}H]noradrenaline in the rat heart during increased sympathetic nervous activity associated with cold. To gain insight into the mechanism involved, the effect of various drugs on the retention of [^{3}H]noradrenaline during increased sympathetic nervous activity was examined.

Male albino rats of the Holtzman strain, 200–225 g, were used. To elicit sympathetic stimulation, rats placed in individual cages, were exposed to cold for 6 hr at 4°. Exposure to cold is known to result in increased sympathetic nervous activity and release of catecholamines (Euler, 1956).

(±)-Noradrenaline-[7-^{3}H]hydrochloride (specific activity 0·040–0·05 mc/mg) obtained from New England Nuclear Corporation was diluted to 6·5 μc/ml with isotonic saline before each experiment. To stimulate sympathetic nervous activity, rats were exposed to cold for 5 hr. Thereafter they were injected with 6·5 μc/100 g with [^{3}H]noradrenaline. The animals remained a further 1 hr in

the cold. Thereafter the rats were killed by a blow at the base of the neck. The hearts were quickly removed, rinsed in ice-cold saline and homogenized in 10 ml 0·4N perchloric acid. The catecholamines were isolated from the clear supernatant fluid of the tissue homogenate by absorption on alumina and the eluates were assayed for tritiated (Whitby, Axelrod & Weil-Malherbe, 1961) and endogenous noradrenaline (Anton & Sayre, 1962).

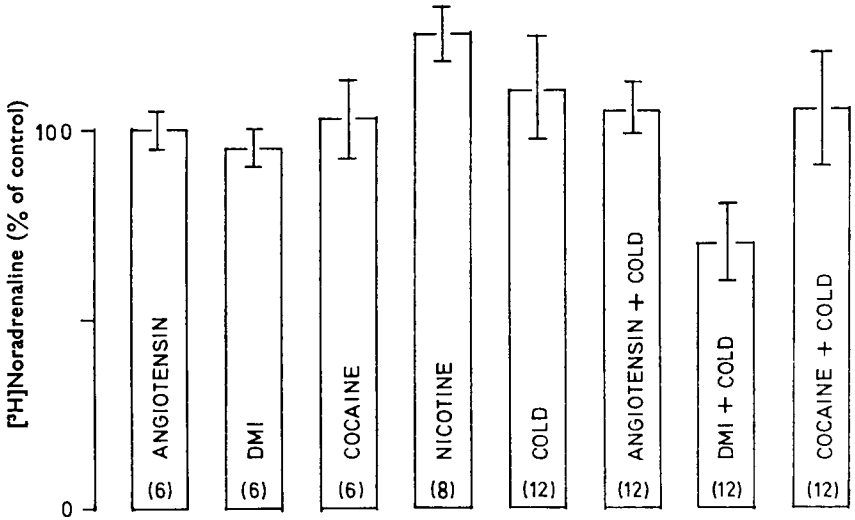

FIG. 1. Effect of various drugs on [³H]noradrenaline content of rat heart during exposure to cold. Rats were exposed to cold for 5 hr. Thereafter, they were injected subcutaneously with 6·5 μc/100 g of [³H]noradrenaline. Animals remained a further 1 hr in the cold. Some animals were administered subcutaneously angiotensin (5 μg/kg), desipramine (DMI) (2·5 mg/kg) or cocaine (10 mg/kg) 10 min after [³H]noradrenaline. All animals were killed and their hearts analysed for [³H]noradrenaline. Each bar represents the mean percentage of [³H]noradrenaline in control animals ± s.e. Numbers in parentheses indicate the number of animals on which each mean is based.

The results (Fig. 1) indicate that there was no significant increase in accumulation of [³H]noradrenaline injected during increased sympathetic nervous activity associated with cold, compared with that of controls. However, under similar conditions, there was a 20–30% release of [³H]noradrenaline when endogenous noradrenaline stores were labelled 1 hr before the experiment. It is, therefore, suggested that retention of [³H]noradrenaline is increased during increased sympathetic activity. In another series of experiments nicotine, a ganglionic stimulant, was used to stimulate sympathetic nervous activity. Rats were injected with 6·5 μc/100 g of [³H]noradrenaline. Nicotine was administered subcutaneously every 2 min.

Animals were killed 1 hr after the injection of [³H]noradrenaline and their hearts were assayed for radioactivity. The results indicate (Fig. 1) that there was a significant increase in retention of [³H]noradrenaline. But nicotine has a dual action, initially it stimulates and then blocks the ganglion cell. Since ganglionic blockade results in increased retention of injected noradrenaline (Bhagat, 1963;1967), results with nicotine may not be due to increase in sympathetic nervous activity, and therefore cannot be considered as conclusive.

The accumulation of [³H]noradrenaline was unaltered by angiotensin or

TABLE 1. EFFECT OF TYRAMINE ON [^{3}H]NORADRENALINE CONTENT OF RAT HEART DURING EXPOSURE TO COLD

Treatment	Noradrenaline		Specific activity
	Endogenous μg/g	[^{3}H] counts/min/g	counts/min/μg
None	0·93 ± 0·03	2087 ± 60	2081 ± 181
Tyramine	0·61 ± 0·05	1174 ± 65	1825 ± 190
Exposure to cold	0·68 ± 0·04	1923 ± 233	2487 ± 203*
Exposure to cold + tyramine	0·39 ± 0·02	936 ± 75	2414 ± 210†

Animals (groups of 6) were exposed to cold at 4° for 5 hr before subcutaneous injection of [^{3}H]noradrenaline 6·5 μc/100 g of body weight, and remained in cold 1 hr more after the injection. Tyramine hydrochloride (10 mg/kg) was injected intramuscularly 30 min after labelled noradrenaline. One hr after the injection of [^{3}H]noradrenaline rats were killed and their hearts were assayed for endogenous and labelled noradrenaline.

* Specific activity of control vs. cold exposed rats $P < 0.05$.

† Specific activity of tyramine treated *vs.* cold + tyramine treated rats $P < 0.05$.

cocaine. Desipramine significantly decreased the accumulation of [^{3}H]noradrenaline during increased sympathetic nervous activity. While both cocaine and desipramine inhibit the uptake of noradrenaline, it seems that they act at different sites or in different ways. It is also possible that the increased retention of [^{3}H]noradrenaline during increased sympathetic nervous activity and the re-uptake of released noradrenaline are different processes. Iversen,Glowinski & Axelrod (1965) also found differences between the action of these two drugs on the uptake of noradrenaline in reserpine-pretreated rats.

Tyramine (Table 1) was almost as effective in releasing noradrenaline in rats no matter whether rats were exposed to cold or not. However, there was an increase in the specific activity of noradrenaline recovered from the heart of rats exposed to cold and injected with tyramine. These findings indicate an increase in retention of [^{3}H]noradrenaline during continuous nerve stimulation.

It is concluded from these results that during increased sympathetic nervous activity there is increased retention of noradrenaline to meet the need for replacement of catecholamines in the nerve endings.

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