Br. J. Pharmac. (1969), 37, 24–33.

Factors involved in maintenance of cardiac catecholamine content: relative importance of synthesis and re-uptake

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1. When animals were exposed to a temperature of 4° C for 6 hr, endogenous catecholamines remained unaltered or reduced slightly depending upon the strain of rats used. In contrast, labelled noradrenaline declined rapidly, but the decline was inhibited when animals were pretreated with monoamine oxidase inhibitors.

2. Increased sympathetic nervous activity associated with cold resulted in a four-fold increase in rate of synthesis of noradrenaline.

3. Reduction in endogenous and labelled catecholamine levels associated with cold was exaggerated by pretreatment with cocaine, imipramine or phenoxybenzamine—drugs known to inhibit the uptake of noradrenaline into the nerve terminal.

4. Cocaine and imipramine in higher doses inhibited the release of both endogenous and labelled noradrenaline, suggesting a dual action: in small doses they increased the depletion of catecholamines by blocking the reincorporation, while in higher doses they inhibited the release of noradrenaline.

5. It is concluded that, under normal conditions, the re-uptake mechanism may not play a significant role in the maintenance of normal cardiac catecholamine levels and that such levels are maintained by synthesis alone. However, when the heart is subjected to high impulse nerve activity, synthesis is not sufficiently accelerated to compensate for impulse-induced massive release and may require the support of an additional mechanism, such as the partial reincorporation of released transmitter. In fact, the re-uptake mechanism is enhanced during high impulse activity.

It is now well-known that noradrenaline is the major transmitter at post-ganglionic sympathetic nerve endings (von Euler, 1956). The transmitter is stored in dense core vesicles which are also the site of its synthesis and from which it is released in response to nerve stimulation. A portion of the released transmitter is destroyed enzymatically while another part is reincorporated into the stores and the remainder

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diffuses away from the site of liberation (Gillespie & Kirpekar, 1965). Reincorporation of noradrenaline into the nerve endings appears to be the most important mechanism for rapid inactivation of the locally released hormone (Gillespie & Kirpekar, 1965). 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.

Normally, tissue noradrenaline remains at a steady level characteristic of each organ. There is considerable evidence that prolonged sympathetic nerve stimulation fails to deplete and in some cases even increases the endogenous noradrenaline of innervated organs (Luco & Goni, 1948 ; von Euler & Hellner-Bjorkman, 1955). Increased sympathetic activity results in an increased synthesis of noradrenaline (Olivario & Stjärne, 1965 ; Fredholm & Sedvall, 1966 ; Gordon, Spector, Sjoerdsma & Udenfriend, 1966 ; Bhagat, 1967). Thus, local synthesis and re-uptake of released transmitter balance the transmitter losses induced by impulse discharge and intraneuronal metabolism and maintain the endogenous levels of noradrenaline in tissues.

However, the capacity of re-uptake, and the extent to which adrenergic transmitter synthesis can be stimulated during increased impulse activity has not been determined. The present study was undertaken to gain information on this point and to determine the relative importance of re-uptake and synthesis in maintenance of cardiac catecholamine levels.

Methods

Male rats weighing 150–200 g were used in all experiments. Various strains of white male rats were used, but care was taken to use the same strain for each set of experiments. The animals were killed by a blow at the base of the neck and decapitated. The hearts were removed and rinsed in ice-cold saline. The tissue was homogenized in 10 ml. of ice-cold 0·4 N perchloric acid. The catecholamines were isolated from the clear supernatant fluid of the tissue homogenates by absorption on alumina and elution with 0·04 N perchloric acid (Anton & Sayre, 1962). The noradrenaline was converted to a trihydroxyindole through oxidation by potassium ferricyanide at pH 6·5 according to the method of von Euler & Lishajko (1961). A 1·0 ml. aliquot of the alumina eluate was added to 10 ml. of a naphthalene dioxane counting solution and this was counted in a liquid scintillation spectrometer. All values reported for ^{3}H-noradrenaline in this paper are uncorrected for the degree of recovery, which ranged from 66 to 80% of the added counts. The specific activity of noradrenaline in tissues was calculated by dividing the radioactivity found in the alumina eluate by the quantity of noradrenaline found in the same eluate. The specific activity was plotted against time on the semi-log paper and method of least square was used to calculate the line of best fit. Turnover rates were estimated from the slopes of decline in specific activity of noradrenaline with time according to the method of Brodie, Costa, Dlabac, Neff & Smookler (1966).

Exposure to cold

Rats were placed in individual wire cages in a cold room at 4° C.

Adrenal demedullation

Demedullation of adrenal glands was carried out under pentobarbital anaesthesia (50 mg/kg intraperitoneally). The capsule of the adrenal was incised at the upper

pole of the gland and the medulla and much of cortical parenchyma were then removed by gently squeezing the organ. Sham operation differed only in that the glands were not disturbed. All animals were used for experiments 96 hr after the operation.

The following substances were used: (±)-noradrenaline-7 ^{3}H (5 c/m-mole, New England Nuclear Corporation, Boston, Mass.), pheniprazine hydrochloride, pargyline hydrochloride (Eutonyl, MO-911), nicotine hydrochloride, cocaine hydrochloride, imipramine hydrochloride, phenoxybenzamine hydrochloride. The dosages of all drugs are expressed in terms of the salt. Details of dosages, time schedule and route of administration are given in the appropriate places under **Results.**

Statistical calculations (*t* test) were performed according to Snedecor (1956).

Results

Effect of cold exposure on the concentration of catecholamines in the rat heart

Exposure to cold results in increased sympathetic nervous activity and release of noradrenaline (von Euler, 1956). Since exposure to cold was to be employed as a means of eliciting sympathetic stimulation, it was considered necessary to establish certain quantitative features of the response of the rat to cold. To obtain the time course of the decline of specific activity in the heart following exposure to cold, rats were injected with ^{3}H-noradrenaline (2·5 μc/100 g, intravenously) 1 hr before the experiment. Cardiac noradrenaline and ^{3}H-noradrenaline were then determined at various time intervals. There was no change in endogenous catecholamine content of the heart at 3 hr, but at 6 hr endogenous levels fell by 29% to 35% in O'Grady rats. The reduction in endogenous levels was not found in all strains of rats used (Table 1). It seems that some rats are more sensitive to cold than others. The adrenal demedullation of rats did not alter the effect of cold on endogenous levels of catecholamines in the heart. The mean concentration in the hearts of ten demedullated rats (Holtzman strain) exposed to cold was 0·73 (S.E. ±0·04) μg/g. The comparable value obtained for eight sham operated rats exposed to cold was 0·71 (S.E. ±0·05) μg/g. The difference between the means is not significant. The levels of ^{3}H-noradrenaline in the hearts of animals exposed to cold fell rapidly (Fig. 1). Estimates of the rates of noradrenaline synthesis in rats (Holtzman strain) showed a four-fold increase in the formation of noradrenaline in the hearts of animals exposed to cold over those of controls. The calculated turnover rate in such animals was 0·19 μg/g per hr and the rate constant of ^{3}H-noradrenaline loss (k) was 0·301 hr^{-1}.

TABLE 1. *Effect of increased sympathetic activity on the levels of cardiac catecholamine*

Strain of rats	Catecholamine concentration in fresh tissue Mean±S.E.M.	
	Control μg/g	Cold μg/g
Sprague Dawley	0·80±0·04(5)	0·86±0·03(5)
Holtzman	1·01±0·04(6)	1·03±0·02(6)
O'Grady	0·70±0·05(10)	0·49±0·02(10)*

* $P<0·01$, control versus cold exposed.
Various strains of rats were placed in individual cages and exposed to 4° C for 6 hr. while control animals were kept at 25° C. Thereafter, rats were killed and their hearts assayed for endogenous noradrenaline.

Effect of monoamine oxidase inhibitors on the concentration of cardiac catecholamines in rats exposed to cold

Monoamine oxidase inhibitors have been shown to block the release of catecholamines by reserpine (Bhagat, 1963) and by guanethidine (Bhagat & Shideman, 1963). Therefore, it was considered of interest to determine whether or not monoamine oxidase inhibitors antagonize the release of cardiac catecholamines associated with cold. Rats (Holtzman strain) received either pheniprazine (10 mg/kg, intramuscularly) or pargyline (25 mg/kg, intramuscularly) 18 hours before the intravenous

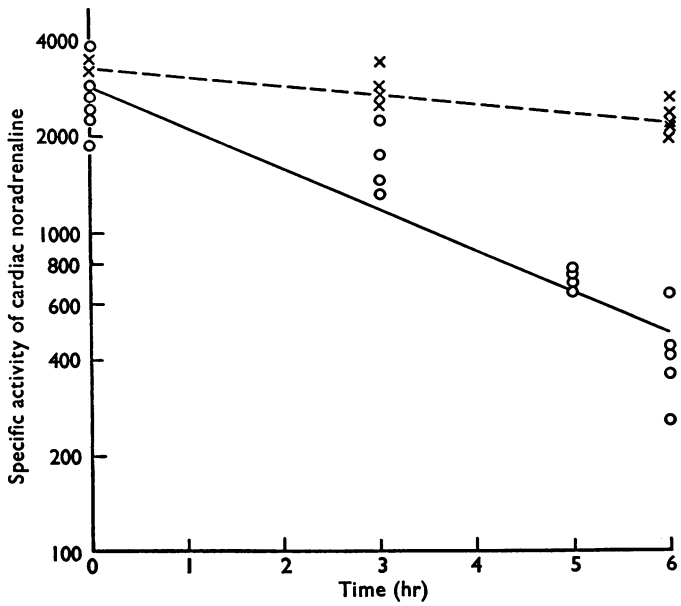

FIG. 1. Effect of cold exposure on the semilogarithmic decline of the specific activity of noradrenaline in rats. Rats (Holtzman strain) were given 2·5 μc/100 g body weight of ^{3}H-noradrenaline intravenously, 1 hr before exposure to cold at 4° C. Animals were killed at various times thereafter, and their hearts were assayed for endogenous and ^{3}H-noradrenaline. The specific activity of noradrenaline was plotted against time on a semilogarithmic paper. Method of least squares was used to calculate the line of best fit. X, Control animals; O, test animals. (The units of specific activity express the ratio of c.p.m./μg noradrenaline.)

TABLE 2. *Effect of cold on the levels of cardiac catecholamines in rats pretreated with monoamine oxidase inhibitors*

	Endogenous Mean ± S.E.M.		^{3}H-noradrenaline Mean ± S.E.M.	
	25° C	4° C	25° C	4° C
Treatment	(μg/g)		(cpm/g)	
None	1·02±0·03	1·05±0·03	1215±21	684±16
Pheniprazine	1·40±0·05*	1·44±0·05*	1390±24*	1077±20*
None	1·02±0·05	1·02±0·03	1188±25	624±20
Pargyline	1·82±0·06*	1·62±0·06*	1407±43*	927±21*

* $P<0·01$, control versus monoamine oxidase inhibitor treated animals.
Groups of five rats (Holtzman strain) were injected with either pheniprazine (10 mg/kg intramuscularly) or pargyline (25 mg/kg intramuscularly) 18 hr before the intravenous administration of labelled noradrenaline (10 μc/100 g). One hour later animals were exposed to cold at 4° C for 6 hr. Animals were killed and their hearts were assayed for endogenous and labelled noradrenaline.

administration of ^{3}H-noradrenaline (10 μc/100 g). One hour later, animals were exposed to cold for 6 hr. Animals were then killed and the concentrations of labelled and endogenous noradrenaline in the hearts was examined. The results of these experiments are summarized in Table 2. Treatment with these drugs inhibited release of ^{3}H-noradrenaline following exposure to cold.

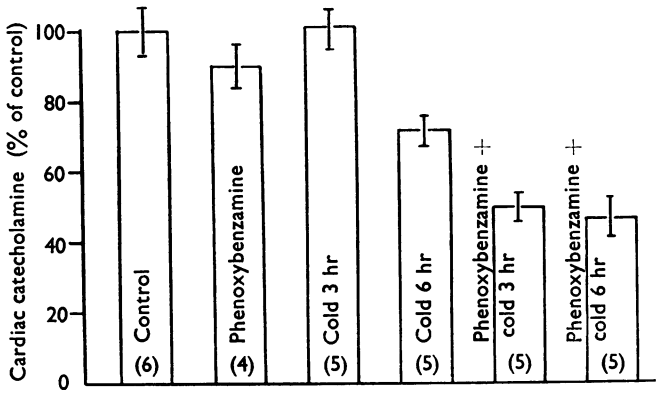

FIG. 2. Effect of phenoxybenzamine on the levels of cardiac noradrenaline in rats exposed to cold. Rats (O'Grady strain) received phenoxybenzamine (25 mg/kg, intramuscularly) and were placed in individual wire cages and exposed to 4° C for 3 and 6 hr while control animals were kept at 25° C. Thereafter, ventricles were assayed for endogenous noradrenaline. Each bar represents the mean percentage of control noradrenaline levels and the vertical lines indicate S.E.M. Numbers in parentheses indicate the number of animals on which each mean is based.

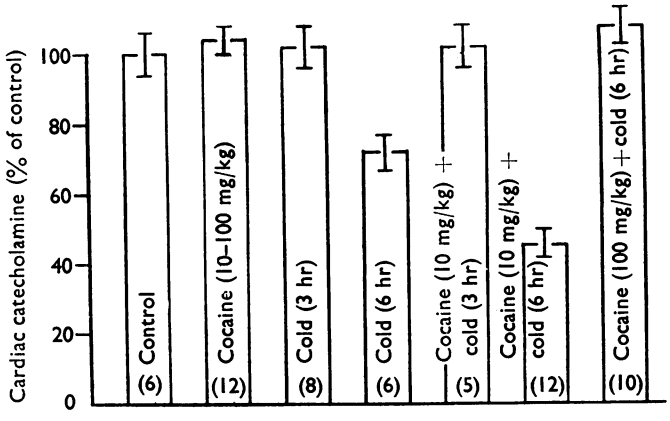

FIG. 3. Effect of various doses of cocaine on the levels of cardiac catecholamines in rats exposed to cold. Rats (O'Grady strain) received various doses of cocaine intramuscularly and were placed in individual wire cages. The animals were exposed to 4° C for 3 or 6 hr, while control animals were kept at 25° C. Thereafter, animals were killed and their ventricles were analysed for noradrenaline content. Each bar represents the mean percentage of control noradrenaline levels and the vertical lines indicate S.E.M. Numbers in parenthesis indicate the number of animals on which each mean is based.

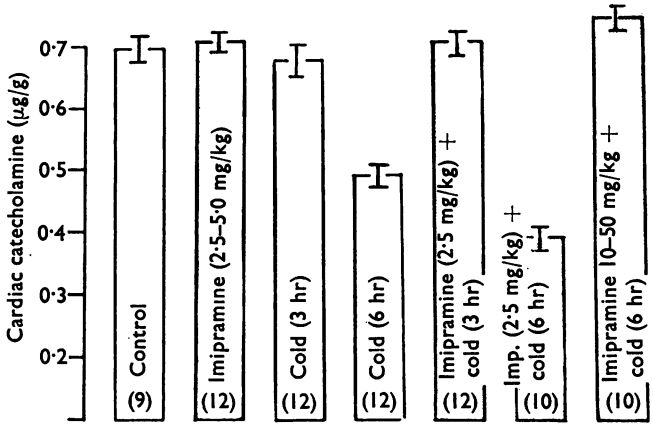

FIG. 4. Effect of various doses of imipramine on the levels of cardiac catecholamines in rats exposed to cold. Rats (O'Grady strain) received various doses of imipramine intramuscularly and were placed in individual wire cages. The animals were exposed to 4° C for 3 or 6 hr, while control animals were kept at 25° C. Thereafter, animals were killed and their ventricles were assayed for noradrenaline content. Each bar represents the mean noradrenaline levels and the vertical lines indicate S.E.M. Numbers in parentheses indicate the number of animals on which each mean is based.

TABLE 3. *Effect of cold exposure on concentrations of ^{3}H-noradrenaline in rat hearts pretreated with cocaine or imipramine*

Treatment	Dose (mg/kg)	^{3}H-noradrenaline Mean ± S.E.M. 25° C	4° C
		c.p.m./g wet tissue	
None	—	668±44 (10)	298±15 (8)
Cocaine	10	702±21 (6)	236±18 (6)*
None	—	700±25 (6)	325±24 (12)
Imipramine	2·5	687±30 (6)	233±14 (8)*
Imipramine	5·0	654±21 (8)	278±19 (8)*

* $P<0{\cdot}01$, control versus drug treated.
Rats (O'Grady strain) were given 20 μc of (±)-noradrenaline-^{3}H intravenously 1 hr before the intramuscular administration of cocaine or imipramine. They were then placed in individual wire cages, and were kept in a cold room at 4° C, while control animals were kept at 25° C. Six hours later animals were killed and ventricles were assayed for labelled noradrenaline.

TABLE 4. *Inhibition of release of ^{3}H-noradrenaline during exposure to cold by cocaine or imipramine*

Treatment	Dose (mg/kg)	No. of Animals	^{3}H-noradrenaline (% of control)
None	—	14	100±2·2
Exposure to cold	—	14	45±1·9*
Cocaine + cold	100	10	114±2·1†
Imipramine + cold	10	10	74±2·4†
Imipramine + cold	50	10	118±4·3†

* $P<0{\cdot}01$, cold exposed versus control
† $P<0{\cdot}001$, drug + cold treated animals versus cold exposed animals.
Rats (O'Grady strain) were given 10 μc of (±)-noradrenaline-^{3}H per 100 g intravenously 1 hr before the intramuscular administration of cocaine or imipramine. They were then placed in individual wire cages and kept at 4° C, while control animals were kept at 25° C. Six hr later, animals were killed and ventricles were assayed for labelled noradrenaline.

Effect of inhibition of re-uptake of released catecholamines by phenoxybenzamine, cocaine or imipramine on the levels of cardiac catecholamines of rats exposed to cold

In order to gain information on the importance of re-uptake of released catecholamines in replenishment of cardiac stores during increased sympathetic nervous activity associated with cold, the following experiments were performed. Rats (O'Grady strain) were injected with phenoxybenzamine (25 mg/kg, intramuscularly), cocaine (10 mg/kg, intramuscularly) or imipramine (2·5 and 5·0 mg/kg, intramuscularly) 15 min before exposure to cold. Animals were killed 3–6 hr thereafter and the concentrations of catecholamines in the ventricles were determined.

Phenoxybenzamine caused a 10–15% depletion of catecholamines, but cocaine or imipramine in the doses employed, did not affect the levels of endogenous cardiac noradrenaline. In the rats exposed to cold, these drugs significantly increased the depletion ($P<0·01$) of cardiac catecholamines associated with cold (Figs. 2, 3, 4). In contrast, at higher doses, both cocaine (100 mg/kg) and imipramine (10–50 mg/kg) antagonized the depletion of cardiac catecholamines ($P<0·01$) associated with cold. The levels of endogenous catecholamines showed an increase rather than a decrease. Higher doses of phenoxybenzamine could not be used because of a 40 to 50% mortality found among these rats when exposed to cold.

In order to confirm these results, experiments were performed similar to those described above except that their catecholamine stores were labelled 1 hr before exposure to cold. The results of these experiments are summarized in Tables 3 and 4. The results confirm that at low doses, both cocaine and imipramine increased the release of ^{3}H-noradrenaline in response to increased sympathetic nervous activity, while at higher doses they inhibited the release of noradrenaline.

Discussion

Exposure to cold is known to result in an increased release of catecholamines from tissue stores (Leduc, 1961), presumably due to increased sympathetic nervous activity, since pretreatment with ganglionic blocking agents prevented release of noradrenaline (Leduc, 1961). Several studies indicate that the noradrenaline released from the heart during cold exposure was derived from all three subcellular fractions: coarse, particulate and soluble (Gutman & Weil-Malherbe, 1967). The decrease in noradrenaline was largest in the coarse fraction and was slight in the particulate fraction.

Vigorous sympathetic nervous activity produced by (1) exposure to cold for 6 hr, (2) administration of drugs such as histamine and β-tetrahydronaphthylamine and (3) by direct sympathetic nerve stimulation for 1 hr, results in increased synthesis of noradrenaline in the heart (Bhagat, 1967). In isolated guinea-pig vas deferens (Weiner & Alousi, 1966 ; Roth, Stjärne & Euler, 1966 ; Weiner & Rabadjija, 1968) and in salivary glands perfused *in situ* (Sedvall & Kopin, 1967) electrical stimulation of the nerves resulted in increased synthesis of noradrenaline in those organs. This increase in synthesis is apparently stimulated at or before tyrosine hydroxylation, the rate-limiting step (Levitt, Spector, Sjoerdsma & Udenfriend, 1965), and not subsequently since noradrenaline-^{3}H formation from dopa-^{3}H was unaltered (Sedvall & Kopin, 1967). Such increase in rates of synthesis keeps pace with the rates of release induced by nerve stimulation and consequently there is only slight or no reduction in catecholamine content of tissues.

In the present study, the results indicate that when rats were exposed to 4° C for 3 hr, there was a significant release of ^{3}H-noradrenaline whereas the endogenous levels of cardiac catecholamine remained unaltered. When the exposure period was increased to 6 hr there was further release of ^{3}H-noradrenaline, but the endogenous levels remained unaffected or decreased by 29% depending upon the strain of rats used. Thus, the relative stability of endogenous noradrenaline in tissues during high impulse activity, associated with cold exposure, accompanied by a release of ^{3}H-noradrenaline, indicates the increase in rate of synthesis. Estimates of rate of synthesis of noradrenaline show that it was 4 times higher in rats exposed to cold than in the control group.

The discrepancy in results observed in different strains of rats used, may be due to the fact that some strains of rats appear to have been more sensitive and increase in the rate of synthesis could not compensate during high impulse activity, resulting in a fall in the level of cardiac catecholamines.

Monoamine oxide plays an important role in the regulation of endogenous amine levels. A considerable amount of noradrenaline is released intraneuronally, metabolized by monoamine oxidase and eliminated from the heart to a great extent. It seems that after inhibition of monoamine oxidase the released noradrenaline is taken up and stored again. Thus, monoamine oxidase inhibitors help to conserve noradrenaline more efficiently, accounting for the slow release of labelled catecholamine during high impulse activity.

Re-uptake of at least some of the nervously released noradrenaline may play a role in the homoeostasis of the neurotransmitter. Prevention of uptake, during high impulse traffic is unlikely. Gillis (1963) reported that labelling of endogenous noradrenaline stores for 3 hr before continuous stimulation of the sympathetic nerves of isolated cat atria for 50 min, at a rate of 10 shock/sec, caused an increase in the specific activity of noradrenaline recovered from the atria. These findings indicate an increase rather than a decrease in uptake during continuous nerve stimulation for a long period. Similarly, Chang & Chiueh (1968) suggested that the uptake mechanism is enhanced on increasing the sympathetic activity. They stimulated the sympathetic nerve to submaxillary gland of rats, intermittently (10 sec/min) at a frequency of 20/sec and found that intermittent stimulation of cervical sympathetic trunk caused an increase of radioactivity in the submaxillary glands by about 100%.

If there is enhancement of re-uptake of released noradrenaline, then it appears most likely that synthesis of noradrenaline was not sufficiently accelerated to balance the increased impulse-induced release. Thus, the decrease in cardiac catecholamine levels observed in the present study in certain strains of rats suggests that there is a definite limit to the extent to which adrenergic transmitter synthesis and the re-uptake mechanism can be stimulated during high impulse traffic.

A disturbance of the re-uptake mechanism would be expected to cause considerable alterations in net release of noradrenaline. Thus, when rats pretreated with phenoxybenzamine, cocaine or imipramine were exposed to cold, levels of cardiac catecholamines (labelled and endogenous) decreased markedly. These drugs have the common property of reducing or abolishing the ability of the tissues to bind noradrenaline in sympathetically innervated organs (Hertting, Axelrod & Whitby, 1961 ; Bhagat & Gilliam, 1965 ; Bhagat, Bovell & Robinson, 1967).

Since these drugs do not interfere with monoamine oxidase or catechol-*o*-methyl transferase activity, it is unlikely that these effects are due to changes in enzymatic degradation of noradrenaline. One interpretation of our results therefore would be that during sympathetic activity most of the discharged noradrenaline was re-captured and incorporated into the post-ganglionic sympathetic nerve endings to conserve transmitter and that this reincorporation was prevented by the drugs (phenoxybenzamine, cocaine and imipramine). Thus, it appears that re-uptake of noradrenaline contributes greatly to the maintenance of the transmitter store during high impulse activity. This suggestion also explains the observation of other workers who found that release of noradrenaline into the venous outflow from the cat spleen increased in the presence of these drugs (Brown, 1960 ; Brown, 1965 ; Gillespie & Kirpekar, 1965).

However, when rats were pretreated with higher doses of cocaine (100 mg/kg) or imipramine (10–50 mg/kg), there was diminished release of labelled noradrenaline and the endogenous levels of noradrenaline showed an increase rather than a decrease, in response to exposure to cold. High doses of these drugs appear to block adrenergic activity and thus inhibit the release of the transmitter. Thus these drugs have a dual action. In low concentrations, both cocaine and imipramine increased the depletion by blocking the reincorporation of the released noradrenaline in response to increased nervous activity, while at higher levels they inhibited the release.

While cocaine (10 mg/kg) or imipramine (5 mg/kg) significantly increased the depletion of cardiac catecholamines in rats exposed to cold at 4° C for 6 hr, they had no effect on the levels of endogenous catecholamine in rats kept at room temperature. Since these drugs act by blocking the re-uptake of released transmitter and since the noradrenaline content in the heart decreases when sythesis of noradrenaline is inhibited at the rate limiting step—that is, tyrosine to dopa (Carlsson, Corrodi & Waldeck, 1963 ; Spector, Sjoerdsma & Udenfriend, 1965 ; Bhagat, 1967), it appears that under resting conditions the re-uptake mechanism does not play a significant role in the maintenance of normal cardiac catecholamine levels and that synthesis alone acts to maintain it. However, this does not appear to be the case when the heart is subjected to high impulse nerve activity. In these circumstances, synthesis is not sufficiently accelerated to compensate for the impulse-induced massive release and may require the support of an additional mechanism—the partial reincorporation of the released transmitter.

A preliminary report of this work was presented at the annual meeting of the Federation of American Societies for Experimental Biology in Atlantic City, April 1968. This work was supported by a grant from Tobacco Research Council U.S.A. We wish to thank Miss K. Hayshida and Neelam Puri for their excellent technical assistance.

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(*Received August 5*, 1968)