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Regulation of metaraminol efflux from rat heart and salivary gland

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1. The turnover rate of noradrenaline (NA) in heart and submaxillary salivary gland was studied in rats exposed to 4° C or maintained at room temperature (22° C). Cold exposure increased the turnover of the NA store in heart but not in salivary gland.

2. In another series of experiments the decline of metaraminol (M) from heart and submaxillary salivary gland was studied in rats exposed to 4° C or maintained at room temperature. Cold exposure accelerated the efflux of M from heart, but not from salivary gland. It is concluded that the accelerated decline of M from heart is the consequence of selective activation of the sympathetic nerves that innervate the heart.

3. The turnover of NA was studied in rat heart after the administration of M (100 μg/kg intravenously) or its precursor α-methyl-meta-tyrosine (200 mg/kg intraperitoneally). Turnover remained essentially normal after these drugs.

4. The administration of desipramine (DMI, 20 mg/kg intraperitoneally) 1 hr after M (100 μg/kg intravenously) induced a rapid sustained efflux of M from heart and salivary gland. The results of this study suggest that the slow decline of M from heart is the result of the great affinity of the amine retrieval mechanism in sympathetic nerve endings for M. DMI inhibits the retrieval mechanism, thus accelerating the efflux of M.

Metaraminol (M), the decarboxylated β-hydroxylated metabolite of α-methyl-meta-tyrosine (α-MMT) is taken up and stored in sympathetic axons (Carlsson & Lindqvist, 1962), where it is probably localized in synaptic vesicles (Vogel, Costa, & Matsumoto, 1965). The intravenous dose of M required to deplete 50% of the noradrenaline (NA) in rat heart is 0·08 mg/kg (Gessa, Costa, Kuntzman & Brodie, 1962). After this dose the concentration of M in the heart declines with a half-time of about 60 hr (Shore, 1966; Costa, Boullin, Hammer, Vogel & Brodie, 1966). Although the sympathomimetic effects of M are weaker than those of NA (Trendelenburg, Muskus, Flemming & Alonso de Le Sierra, 1962) M can replace up to 95% of the cardiac NA store without impairing sympathetic nerve function (Andén & Magnusson, 1965). Because animals have normal sympathetic function

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despite the low concentration of tissue NA, it was suggested that 95% of NA stores are functionally inert (Andén & Magnusson, 1965).

At present it is believed that changes of sympathetic function are more accurately revealed by the turnover rate than by the steady-state level of tissue NA (Brodie, Costa, Dlabac, Neff & Smookler, 1966). Hence a study of the turnover rate of heart NA, when M is present in sympathetic axons, might explain why neurones function normally even though NA stores are depleted by 95%. Crout, Alpers, Tatum & Shore (1964) have suggested that M might be a " false transmitter " because electrical stimulation of sympathetic nerves released M from the perfused cat heart. They inferred that physiological nerve activity increased the efflux of the drug from cardiac storage sites. In support of this hypothesis, it was reported that in rats injected with M, the drug concentrations declined more slowly from decentralized salivary glands than from normal glands (Almgren & Waldeck, 1967).

The neuronal transport system that retrieves amines released by nerve impulses shows a greater affinity for M than for NA (Burgen & Iversen, 1965); therefore, the long half-time of M in the hearts of rats injected with this drug might be related to the great affinity of the retrieval mechanism for M. This inference is supported by the report of Carlsson & Waldeck (1965) that protryptyline blocked the uptake of tritium-labelled NA (^{3}HNA) by sympathetic nerves and accelerated the rate of disappearance of ^{3}H-metaraminol from mouse heart. Our report concerns a study of the temporal changes of M concentrations in various tissues, its relationship to catecholamine turnover, to treatment with desipramine hydrochloride (DMI), and to the functional state of sympathetic neurones.

Methods

The experimental animals were male Sprague-Dawley rats weighing about 200 g each, obtained from New York Breeding Farms, New City, New York. Racemic metaraminol-7-^{3}H HCl (New England Nuclear Corporation), 0·5 μg of base, diluted with non-radioactive (−)-metaraminol, was injected intravenously in doses of 100 μg/kg. In some experiments the precursor of M, (±)-α-MMT (Regis Chemical Company), 200 mg/kg, was injected intraperitoneally 16 hr before killing the animals to allow for the formation and accumulation of M *in vivo*. In one series of experiments DMI 20 mg/kg was administered intraperitoneally 1 hr after M to study its effect on the decline of M concentrations in heart and submaxillary salivary gland.

Metaraminol was extracted from tissues by the method of Shore & Alpers (1964). Tissue concentrations were estimated by measuring the radioactivity in a portion of the final hydrochloric acid fraction in Bray's counting mixture (Bray, 1960) or by forming a fluorophor with *o*-phthaladehyde (Shore & Alpers, 1964). The intensity of the fluorescence in tissue extracts was compared with standards of authentic M.

The turnover rate of endogenous NA was measured by injecting (±)-7-^{3}HNA (10·2 c/m-mole, New England Nuclear Corporation), 5 μc/kg, intravenously and following the change of NA specific activity with time, or by administering (−)-α-methyltyrosine (α-MT) 200 mg/kg, intravenously and following the decline of NA concentrations with time (Brodie *et al.*, 1966). Noradrenaline was estimated as described previously (Neff & Costa, 1966).

Results

The cardiac levels of M and NA were measured at various times after α-MMT (Table 1). Table 1 compares the NA and M content found in rat heart after α-MMT and α-MT with that of rats given only α-MMT. The concentration of M appeared to be identical in the two groups of animals ; however, the NA content declined with an apparent half-time of 2 hr in the rats treated with α-MT (Table 1). Because heart NA concentrations were relatively constant from 16 to 22 hr in the rats treated with α-MMT alone (Table 1), it was presumed that a new steady-state was established where influx and efflux of NA were balanced.

The fractional rate constant of NA efflux (k) was estimated from the equation $k = \frac{0{\cdot}693}{\text{NA } T_{1/2}}$ where NA $T_{1/2}$ is the time required for the NA concentrations to decline by one-half after the α-MT injection. The turnover rate of NA was calculated by multiplying the new steady-state level of heart NA by k (0·16 μg/g × 0·35/hr = 0·056 μg/g per hr). Because the NA concentrations shown in Table 1 were too low for accurate half-life estimation, the NA turnover rate was also estimated by injecting ^{3}HNA and following the change of specific activity with time. These experiments are reported in Table 2. The results of this turnover study were similar to that reported in Table 1. Moreover, Table 2 shows that the turnover rate of cardiac NA is almost identical in control and M treated rats.

TABLE 1. *Concentration of metaraminol (M) and noradrenaline (NA) in rat heart after α-methyl-m-tyrosine (α-MMT) or after α-MMT and α-methyl-p-tyrosine (α-MT)*

Hours after * α-MMT	Amine concentrations in heart after α-MMT		Hours after † α-MT	Amine concentrations in heart after α-MMT and α-MT	
	NA μg/g	M μg/g		NA μg/g	M μg/g
16	0·16 ± 0·013	0·31 ± 0·012	—	0·16 ± 0·013	0·31 ± 0·012
17	—	0·32 ± 0·004	1	0·10 ± 0·011‡	0·33 ± 0·007
18	—	0·33 ± 0·007	2	0·084 ± 0·009‡	0·34 ± 0·006
22	0·18 ± 0·014	0·34 ± 0·006	6	—	0·33 ± 0·001

Data are presented as mean ± S.E. from five animals.

* Rats were injected intraperitoneally with α-MMT (200 mg/kg) and beginning at 16 hr NA and M concentrations determined.

† Rats were injected intraperitoneally with α-MMT (200 mg/kg) and 16 hr later intravenously with α-MT (200 mg/kg). Animals were then killed at the times indicated and NA and M concentrations determined.

‡ $P < 0{\cdot}05$ when compared with rats killed 16 hr after α-MMT.

TABLE 2. *Turnover rate of heart noradrenaline (NA) in control and metaranimol (M) treated rats*

	NA concentration μg/g	Rate constant of decline k hr^{-1}	Turnover rate μg/g per hr
Control	0·87 ± 0·02 (24)	0·064 ± 0·04	0·055
Metaraminol	0·33 ± 0·01 (24)	0·16 ± 0·02	0·052

Rats were injected with M (100 μg/kg intravenously) 20 hr before (±) ^{3}HNA(5 μc/kg intravenously). Turnover rate of NA was estimated by the method of Brodie *et. al* (1966). Data are presented as mean ± S.E., with number of animals in parenthesis.

Table 3 compares the turnover rate of heart and salivary gland NA in rats kept at room temperature (22° C) or at 4° C for 16 hr before estimating the turnover. The NA concentration of either tissue after 16 hr of cold exposure was not significantly different from that of rats kept at room temperature, but the turnover rate of heart NA was increased. In contrast, the turnover rate of NA in salivary gland was not altered by cold exposure (Table 3). At 22° C, the NA in salivary gland turned over at a faster rate than heart NA. Cold exposure and pretreatment with M (Tables 2 and 3) increased the efflux (k) of heart NA but only cold exposure increased its turnover rate. Exposure to cold also increased the rate of M efflux from heart

TABLE 3. *Turnover rate of heart and salivary gland noradrenaline (NA) in rats exposed to different enviromental temperatures*

	NA concentration μg/g		Rate constant of decline k hr^{-1}		Turnover rate (μg/g per hr)	
Tissue	4° C	22° C	4° C	22° C	4° C	22° C
Heart	0·66 ± 0·05	0·73 ± 0·07	0·12 ± 0·03*	0·064 ± 0·007	0·099	0·046
Salivary gland	0·97 ± 0·15	1·04 ± 0·12	0·11 ± 0·03	0·10 ± 0·02	0·10	0·10

Rats were placed at 4° C or maintained at 22° C for 16 hr before an intravenous injection of ^{3}HNA (5 μc/kg). Groups of four to six rats were killed at 2, 4, 14 and 23 hr after injection. Data are presented as mean ± S.E. k was calculated from the exponential decline of NA specific activity (Brodie *et al.*, 1966).

*$P < 0.05$ when compared with rats kept at 22° C.

TABLE 4. *Efflux of metaraminol (M) from heart and salivary gland of rats exposed to different environmental temperatures*

Hours after injection	Heart M* μg/g		Salivary gland M μg/g	
	4° C	22° C	4° C	22° C
2	0·16 ± 0·01†	0·27 ± 0·02	0·41 ± 0·04	0·47 ± 0·04
8	0·041 ± 0·01†	0·19 ± 0·02	0·26 ± 0·02	0·30 ± 0·04
13	0·023 ± 0·004†	0·21 ± 0 02	0·19 ± 0·01	0·23 ± 0·02

Rats were injected intravenously with ^{3}H-metaraminol (100 μg/kg) and immediately exposed to 4° C or maintained at 22° C. The results are calculated on the assumption that only the (−)-metaraminol in the radioactive racemic mixture injected is retained by the tissues (Shore *et al.*, 1964).

* Data are expressed as mean ± S.E. of four observations.

†$P < 0.05$ when compared with the animals kept at 22° C.

TABLE 5. *Acceleration of the rate of metaraminol (M) disappearance from rat salivary gland and heart after desmethylimipramine (DMI)*

	Salivary gland		Heart	
Hours after ^{3}H-metaraminol	Control μg/g ± S.E.	DMI μg/g ± S.E.	Control μg/g ± S.E.	DMI μg/g ± S.E.
4	0·32 ± 0·02	0·27 ± 0·02*	0·21 ± 0·01	0·12 ± 0·07†
8	0·24 ± 0·03	0·17 ± 0·04*	0·20 ± 0·015	0·072 ± 0·005†
22	0·12 ± 0·02	0·032 ± 0·09†	0·09 ± 0·012	0·026 ± 0·002†

Rats were injected with ^{3}H-metaraminol (100 μg/kg intravenously) and 1 hr later given DMI (20 mg/kg intraperitoneally) or maintained as control animals. Groups of at least four rats were killed at various times after metaraminol. Data are expressed as mean ± S.E. The results are calculated on the assumption that only the (−)-metaraminol in the radioactive racemic mixture injected is retained by the tissues.

*$P < 0.05$ when compared with animals given only metaraminol.

†$P < 0.01$ when compared with animals given only metaraminol.

(Table 4). The rate of efflux of M from salivary gland was faster than that from heart (Table 4), and was not accelerated by cold exposure.

Table 5 shows that the rate of efflux of M from heart and salivary gland was significantly accelerated by giving DMI 1 hr after M.

Discussion

It has been established that sympathetic nerves take up and store M, but the stoichiometry between the concentrations of M and NA depletion as proposed by some investigators has been challenged. Several laboratories (Shore, Busfield & Alpers, 1964 ; Costa *et al.*, 1966 ; Johnson & Mickle, 1966) have reported that the replenishment of cardiac NA is faster than the rate of decline of M levels. Analogous kinetic inconsistencies have been reported for α-methyl-noradrenaline, another "false transmitter" which in several aspects resembles M. Maitre & Staehelin (1967) explained the lack of stoichiometry between the cardiac concentration of NA and α-methyl-noradrenaline in guinea-pigs injected with α-methyl-3,4-dihydroxyphenylalanine by postulating binding to physiologically inert sites. The great affinity of the neuronal uptake mechanisms for M (Burgen & Iversen, 1965) and the resistance of M to metabolism by monoamine oxidase (Pletscher, Gey & Burkard, 1966) suggest that M may behave kinetically quite unlike endogenous NA. Crout *et al.* (1964) have proposed that M is initially localized in axonal sites that are readily accessible to release by nerve impulses, later the amine moves to a compartment which is functionally inert.

Our data and those of Johnson & Mickle (1966) and Johnson & Pugsley (1968) suggest that M is released by nerve activity and that the accelerated rate of M disappearance from hearts of rats exposed to cold (Table 4) is probably related to increased activity of sympathetic neurones. In support of this thesis, a comparison of the rate of efflux of M from salivary gland and heart of rats exposed to cold is presented in Table 4. During cold exposure, the decline of M concentrations is significantly accelerated in heart, but not in salivary gland. Similarly, cold exposure accelerated NA turnover in heart, but not in the salivary gland (Table 3).

After monoamine oxidase inhibitors, brain NA accumulates at similar rates in normal and α-MMT treated rats (Costa, Gessa, Hirsch, Kuntzman & Brodie, 1962), indicating that despite the displacement of endogenous NA by M, the amine does not affect the biosynthesis of NA. Tables 1 and 2 also demonstrate that the turnover rate of cardiac NA is not significantly changed by the presence of M. It is currently believed that catecholamine levels modulate their own synthesis rate by end product inhibition (Neff & Costa, 1966 ; Spector, Gordon, Sjoerdsma & Udenfriend, 1967 ; Ngai, Neff & Costa, 1968). The findings reported in Tables 1 and 2 seem to oppose this hypothesis because the levels of heart NA are decreased while the turnover rate of heart NA remained unchanged. The low concentrations of cardiac NA (Tables 1 and 2), however, are associated with the presence of sizable amounts of M (Tables 1, 4, 5). It is possible that the latter amine prevents the acceleration of NA synthesis when the NA concentrations are low. This is not improbable, as a recent report indicated that high concentrations of tyramine, a chemical analogue of M, could inhibit NA synthesis (Weiner & Selvaratnam, 1968).

In keeping with the current belief that sympathetic function is related to NA turnover, the data of Tables 1 and 2 may explain why sympathetic function appears

normal after M treatment. When heart NA is depleted by M, the turnover rate of NA is normal, suggesting that synthesis is sufficient to maintain sympathetic function. Apparently, newly synthesized NA does not displace M from binding sites in synaptic vesicles but mixes with the small NA compartment still remaining in the axon and is subsequently utilized in axonal function. This inference is in keeping with the finding that after M the average life time of an NA molecule (calculated as the reciprocal of k) in heart is reduced by about 66%.

When the splenic nerves are electrically stimulated the amount of NA found in the perfusion fluid of the isolated spleen increases when the rate of stimulation changes from 10 to 30 Hz (Brown & Gillespie, 1957). Similar results have been interpreted by others (Folkow, Haggendal & Lisender, 1967) to indicate that high frequency stimulation reduces the efficiency of the NA retrieval mechanism. Folkow *et al.* (1967) estimated that at physiological rates of nerve stimulation, *i.e.*, up to 8 Hz, about 80% of the released transmitter is retrieved by sympathetic nerves. When the rate of stimulation is increased, the percentage retrieved is proportionally diminished. To test whether or not the retrieval of M, like that of NA, is a frequency dependent phenomenon, we compared the rate of disappearance of M from salivary gland and heart in cold exposed rats and in rats maintained at room temperature. As shown in Table 3, cold exposure increased the turnover rate of cardiac NA but not the turnover rate of NA in salivary gland. This observation is consistent with the view that cold exposure increases nerve traffic in the sympathetic nerves innervating the heart (Oliverio & Stjärne, 1965 ; Gordon, Spector, Sjoerdsma & Udenfriend, 1966), but not the salivary gland. Moreover, the differences between the turnover of NA in heart and salivary gland implies that the increase of the turnover rate of cardiac NA is a local phenomenon, probably related to the function of sympathetic axons innervating the heart. The data presented in Table 4 show that exposure to cold increased the rate of M efflux from heart, but not from the salivary gland. Because the reuptake mechanism is less efficient when neuronal activity increases (Folkow *et al.*, 1967) it might be inferred that the high affinity of the reuptake mechanisms for M (Burgen & Iversen, 1965) is a factor in determining the long half-life of cardiac M.

There is convincing evidence that tricyclic antidepressants inhibit the uptake of NA and M by sympathetic axons (Dengler, Spiegel & Titus, 1961 ; Carlsson, Fuxe, Hamberger & Lindqvist, 1966). If this uptake mechanism controls the rate of efflux of M, then tricyclic antidepressants should shorten the half-life of M in heart and salivary gland. Carlsson & Waldeck (1965) reported that protryptiline caused an abrupt but short lasting increase of the decline rate of ^{3}H-metaraminol from mouse heart. In contrast, in the present study, DMI increased the rate of M efflux from rat heart and salivary gland during several hours. Perhaps species differences in the rate of drug metabolism, differences in the drugs themselves or doses of M administered might explain the discrepancy between the time course of the effect seen in our experiments and those of Carlsson & Waldeck (1965).

The increased rate of loss of M from heart and salivary gland (Table 5) after DMI treatment supports the hypothesis that the biological half-life of M depends on its retrieval by an amine pump. Moreover, these data favour the view that the rate of M loss is directly related to neuronal activity.

In conclusion, our results show that: (1) the turnover rate of endogenous NA may explain why sympathetic function is maintained despite the depletion of heart

NA after M treatment; (2) the turnover rate of NA is not increased above normal after M treatment, suggesting that end product control of NA synthesis is masked when M is localized in sympathetic nerves; (3) the rate of M efflux from heart and salivary gland can be increased when monoamine uptake by neurones is prevented by DMI; (4) cold exposure, which enhanced the turnover rate of cardiac NA, but not that of salivary gland, increased the efflux of M from heart, but not from salivary gland.

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