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The formation and release of metaraminol during exposure to warm or cold environments

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1. Rats were injected intraperitoneally with alpha-methyl-*m*-tyrosine (400 mg/kg) and placed at either 27° C or 4° C. The levels of alpha-methyl-*m*-tyramine, metaraminol and noradrenaline were determined in heart tissue after 1, 4 and 12 hr of treatment. The excretion of metaraminol, alpha-methyl-*m*-tyramine, noradrenaline, adrenaline and 3-methoxy-4-hydroxyphenylglycol (MHPG) was also estimated in both treated and control rats.

2. Cold exposure increased both the formation and excretion of metaraminol. Hearts removed from the cold-stressed rats 4 hr after injection contained significantly more metaraminol than hearts taken from animals maintained in the warm environment. Twelve hours after treatment, no metaraminol remained in the hearts of cold-exposed rats, whereas significant quantities of the amine still remained in the hearts of rats kept at 27° C. These results support the false transmitter concept advanced for metaraminol as they demonstrate that *in vivo* sympathetic stimulation can increase both the formation and release of metaraminol.

3. Alpha-methyl-*m*-tyrosine produced a greater fall in cardiac noradrenaline in the rats kept at 27° C. Whereas an approximate mole-for-mole replacement of metaraminol for noradrenaline existed at 27° C, no such relationships existed at 4° C. Twelve hours after treatment the hearts of cold-stressed rats contained no metaraminol and only 40% of control noradrenaline levels. These results do not support the necessity for a mole-for-mole replacement of noradrenaline with metaraminol to produce a catecholamine loss.

4. Alpha-methyl-*m*-tyrosine depressed the noradrenaline excretion for at least 24 hr in the cold-stressed rats. Excretion of 3-methoxy-4-hydroxyphenylglycol was also lower in the treated rats between 0 and 12 hr in the cold but rose abruptly between 12 and 24 hr to exceed the quantity excreted by the control animals. This increase suggests an increase in noradrenaline synthesis, which may be related to the depletion of metaraminol from the body.

5. The results of this paper support the postulate that metaraminol may function as a false transmitter. They do not agree with the concept that the loss of noradrenaline from tissue sites is dependent upon a mole-for-mole replacement with metaraminol.

Carlsson & Lindqvist (1962) reported a fall in tissue noradrenaline levels following the administration of alpha-methyl-*m*-tyrosine and its conversion into metaraminol. Subsequent workers demonstrated that metaraminol replaces, at least partially,

the noradrenaline lost and is itself bound within the nerves (Shore, Busfield & Alpers, 1964; Andén, 1964; Udenfriend & Zaltzman-Nirenberg, 1964). The metaraminol stored in sympathetic nerves may be released either by catecholamine-releasing drugs or by *in vitro* or *in vivo* sympathetic stimulation (Crout, Alpers, Tatum & Shore, 1964; Johnson & Mickle, 1966). It has therefore been postulated that metaraminol can serve as a false transmitter. In order to satisfy fully this interesting postulate, however, evidence must be provided that conditions which normally increase the synthesis and secretion of noradrenaline also increase the formation and release of metaraminol. The present research was undertaken to support or refute the possibility that sympathetic stimuli can increase the formation and release of metaraminol.

Leduc (1961) demonstrated that exposing rats to 4° C increases the excretion of noradrenaline. Other workers (Drazkóczy, Pulley & Burack, 1966; Maickel, Matussek, Stern & Brodie, 1967; Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Oliverio & Stjärne, 1965) showed that exposure to cold increases the synthesis of noradrenaline. To satisfy the false transmitter concept exposure to cold must also increase the formation and release of metaraminol. The possibility was investigated by treating rats with alpha-methyl-*m*-tyrosine, placing them at 27° C or 4° C, and measuring cardiac levels and urinary excretion levels of alpha-methyl-*m*-tyramine and metaraminol. The influence of metaraminol on normal catecholamine synthesis, storage and excretion was also studied by measuring tissue levels of noradrenaline and the excretion of adrenaline, noradrenaline and 3-methoxy-4-hydroxyphenylglycol (MHPG).

Methods

Male Wistar rats, 200–300 g, obtained from Canadian Breeding Laboratories, were used throughout the study.

The rats were injected intraperitoneally with DL-alpha-methyl-*m*-tyrosine (400 mg/kg) or an equivalent volume of the injection vehicle. Solutions for injection were prepared by the addition of the amino-acid to 0.5 M phosphate buffer pH 7.4 and a few millilitres of 5N-NaOH to effect solution. The pH was then adjusted to 7.4 by the addition of 1N-HCl. Immediately following treatment the rats were divided into two groups. One group was placed at 27° ± 1° C while the second group was transferred to 4° ± 1° C.

The animals were placed in individual wire cages for the collection of urine (Johnson & Mickle, 1966). The rats were killed by decapitation 1, 4 or 12 hr after injection and the hearts quickly removed.

For the determination of metaraminol and alpha-methyl-*m*-tyramine, single rat hearts were homogenized for 5 min in 25 ml. of 0.4 N perchloric acid (VirTis Tissue Homogenizer). Following centrifugation of the extract, the supernatant was neutralized with cold potassium carbonate to pH 6.5 and the precipitate spun off. The remaining solution (approximately 25 ml.), was run through a Dowex-50Wx4 column (200–400 mesh, 0.6 cm × 4.5 cm), previously treated as described by Carlsson & Lindqvist (1962). Metaraminol and alpha-methyl-*m*-tyramine were eluted separately with 1N-HCl and 2N-HCl respectively, as previously described (Pugsley & Johnson, 1968). The two amines were then coupled with *o*-phthaladehyde and measured fluorimetrically (Shore & Alpers, 1964). To calculate the percentage

recovery of metaraminol and alpha-methyl-*m*-tyramine, both chemicals were added to hearts in amounts such that the levels were similar to those found after the injection of alpha-methyl-*m*-tyrosine (400 mg/kg). Following the addition of 1–5 μg of metaraminol and 1–5 μg of alpha-methyl-*m*-tyramine, the recoveries of metaraminol and alpha-methyl-*m*-tyramine averaged 60.2% ±5.2% (S.E.) and 62.0% ±4.5% (S.E.) respectively. All results on heart levels are corrected for incomplete recovery.

Cardiac noradrenaline was extracted by homogenization in 0.4 N perchloric acid. Analysis of the hearts was carried out by the method of Euler & Lishajko (1961). Four hearts were pooled for each set of noradrenaline determinations. A 95% recovery of noradrenaline was obtained.

Urine samples were collected for three periods, 0–4 hr, 4–12 hr and 12–24 hr after treatment. Urine was analysed for metaraminol and alpha-methyl-*m*-tyramine by the method of Pugsley & Johnson (1968), adrenaline and noradrenaline according to the technique of Euler & Lishajko (1961). 3-Methoxy-4-hydroxyphenylglycol was determined by a modification of the method of Ruthven & Sandler (1965). A 5 ml. urine sample made up to 10 ml. with 0.1 M citrate buffer pH 6.5 was used instead of a 10 ml. urine sample as in the Ruthven & Sandler method. After hydrolysis, 0.1 ml. of a solution of ethylenediamine tetraacetic acid (100 mg/ml.) and 1 mg of ascorbic acid were added to the samples, which subsequently were adjusted to pH 6.0 with 5N-K_2CO_3 and 1N-HCl. The samples were then passed through columns of Amberlite CG 120 Type II cation exchange resin at a speed of approximately 10 ml./30 min. Evaporation and oxidation were then carried out to estimate MHPG according to the Ruthven & Sandler method. Recoveries of the various chemicals in the urine were not determined and the levels should not be taken as absolute values.

Results

Both control and treated rats seemed to be in good physical condition throughout the experiment.

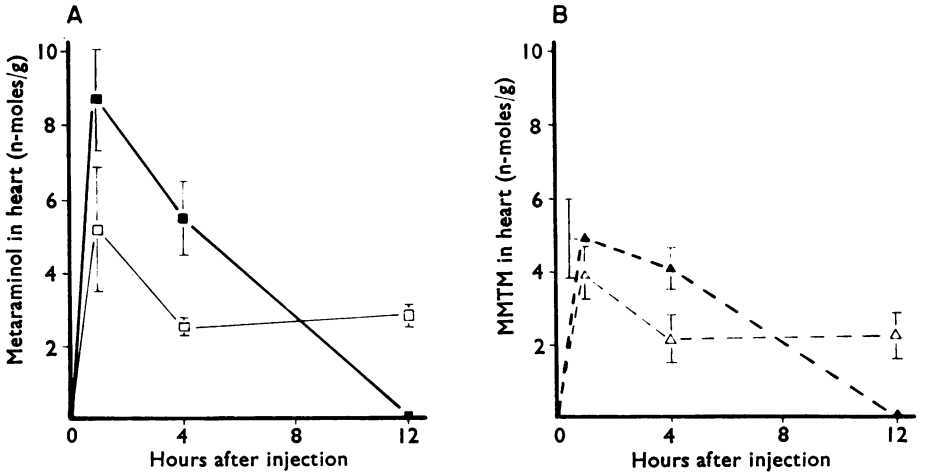

FIG. 1. Concentrations of metaraminol (A) and alpha-methyl-*m*-tyramine (MMTM) (B) in hearts of rats kept at 27° C (□, △) or 4° C (■, ▲) after injection with alpha-methyl-*m*-tyrosine. Points represent means of five to eight rats. Standard errors are plotted for each mean value.

TABLE 1. *Levels of metaraminol (MA), alpha-methyl-*m*-tyramine (MMTA), and noradrenaline (NA) in hearts of rats kept at 27° C and 4° C after injection of alpha-methyl-*m*-tyrosine (MMT) (400 mg/kg)*

	27° C			4° C		
	1 hr	4 hr	12 hr	1 hr	4 hr	12 hr
Metaraminol	5·17±1·70 (5)	2·47±0·22 (8)	2·81±0·33 (5)	8·71±1·37 (5)	5·49±0·99 (8)	0 (5)
α-methyl-*m*-tyramine	3·96±0·73 (5)	2·14±0·66 (8)	2·21±0·63 (5)	4·91±1·09 (5)	4·07±0·57 (8)	0 (5)
NA (MMT) treated	2·46±0·32 (4)	2·10±0·14 (4)	2·05±0·21 (4)	2·10±0·35 (4)	1·66±0·16 (4)	1·65±0·23 (4)
NA (control)	1·31±0·06 (4)	0·92±0·13 (4)	0·02±0·007 (4)	1·07±0·19 (4)	1·08±0·10 (4)	0·63±0·15 (4)

The results for metaraminol and α-methyl-*m*-tyramine are shown in Figs. 1A and B; the rest are shown in Figs. 3A and B.

Values in the table are expressed as n-moles/g of heart tissue. The time intervals indicate the time of sacrifice after injection. Determinations of MA and MMTA were made on single rat hearts. Each value is the mean±S.E.M. of the number of hearts indicated in parenthesis corrected for incomplete recovery and tissue blanks. All determinations of noradrenaline were performed on four pooled hearts and each value represents the mean of four determinations.

TABLE 2. *Urinary levels of noradrenaline (NA), adrenaline (A), 3-methoxy-4-hydroxyphenylglycol (MHPG), metaraminol (MA) and alpha-methyl-*m*-tyramine (MMTA) in rats kept at 27° C and 4° C after injection with alpha-methyl-*m*-tyrosine (MMT) (400 mg/kg)*

	27° C			4° C		
	0–4 hr	4–12 hr	12–24 hr	0–4 hr	4–12 hr	12–24 hr
NA (control)	0·45± 0·12	0·44± 0·09	0·50± 0·17	1·39± 0·14	1·92± 0·30	2·90± 0·08
Na (MMT treated)	0·84± 0·12	0·59± 0·13	0·32± 0·08	1·88± 0·40	1·39± 0·08	1·79± 0·21
A (control)	0·27± 0·13	0·13± 0·09	0·26± 0·16	0·41± 0·09	0·53± 0·21	0·36± 0·03
A (MMT treated)	0·54± 0·16	0·35± 0·10	0·20± 0·10	0·98± 0·15	0·43± 0·14	0·50± 0·17
MHPG (control)	64·55±12·82	156·00±27·49	155·00±34·80	173·33±15·78	177·00±12·00	248·19± 7·62
MHPG (MMT treated)	109·00±17·70	105·24±21·22	57·39±15·20	87·95±22·15	106·65±15·13	301·34± 8·61
Metaraminol	10·24± 2·02	6·86± 0·77	8·92± 1·12	18·67± 1·15	5·77± 1·29	2·52± 0·51
MMTA	415·01±45·86	351·61±13·99	73·86± 6·72	481·48±12·01	238·57± 8·99	81·40±12·37

The results for metaraminol and α-methyl-*m*-tyramine are shown in Figs. 2A and B, those for noradrenaline and adrenaline in Fig. 4, and those for 3-methoxy-4-hydroxyphenylglycol in Fig. 5.

Values in the table are expressed as n-moles/kg per hr. The time intervals indicate the time at which urine is collected. Each value is the mean±S.E.M. of eight to sixteen rats. The metaraminol and alpha-methyl-*m*-tyramine values have been corrected for urine blanks. No correction has been made for percentage recovery of other compounds in urine.

Analysis of hearts taken from animals kept at 27° C and from rats placed at 4° C revealed no significant difference in the concentrations of metaraminol and alpha-methyl-*m*-tyramine between the two groups at the end of the first hour (Table 1, Figs. 1A and B). Four hours after injection, however, significantly more metaraminol and alpha-methyl-*m*-tyramine were found in the hearts of rats exposed to 4° C than in the animals placed at 27° C ($P<0.05$). This pattern was reversed by the end of 12 hr ; neither amine could be detected in the hearts of the cold-stressed rats, whereas significant quantities of each remained in the hearts of the animals exposed to 27° C.

Exposure of the rats to 4° C immediately after the injection of alpha-methyl-*m*-tyrosine increased ($P<0.05$) the excretion of metaraminol during the first 4 hr (Table 2, Fig. 2A). The quantity of metaraminol in the urine subsequently fell

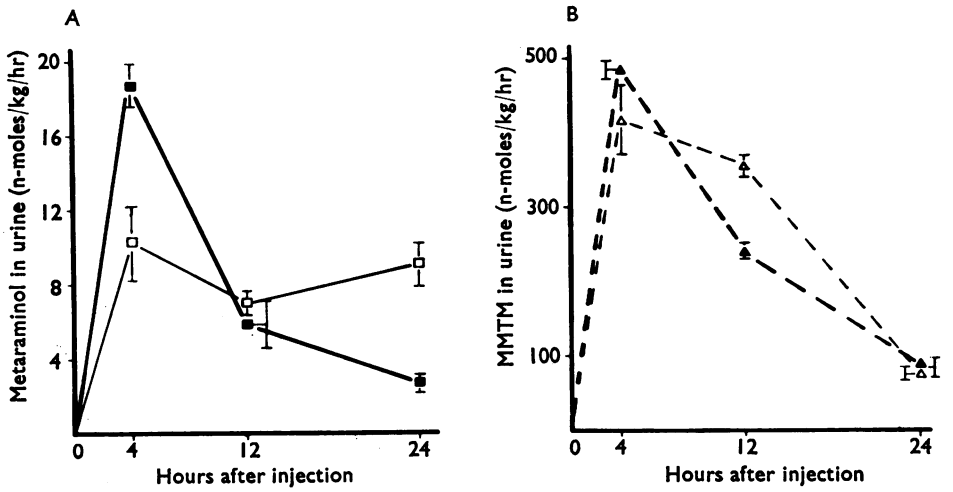

FIG. 2. Urinary excretion of metaraminol (A) and alpha-methyl-*m*-tyramine (B) from rats kept at 27° C (□, △) or 4° C (■, ▲) after injection with alpha-methyl-*m*-tyrosine. Points are means of eight to sixteen rats. Standard errors are plotted for each mean value.

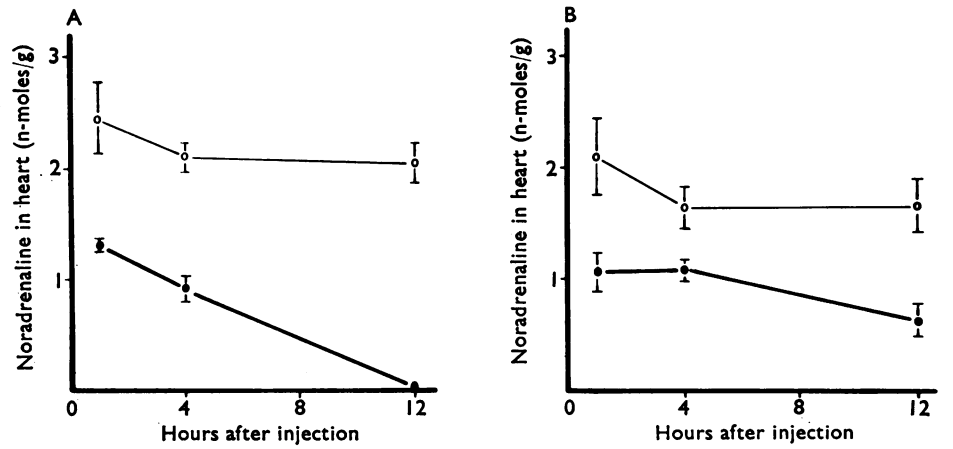

FIG. 3. Concentrations of noradrenaline in hearts of control (○—○) and treated (●—●) rats kept at 27° C (A) or 4° C (B). First plotted values represent levels of noradrenaline 1 hr after injection. Points are means of four values. Standard errors are plotted for each mean value.

markedly, so that between 12 and 24 hr the cold-stressed animals excreted less of the amine ($P<0.05$) than the rats exposed to 27° C. Cold exposure did not significantly increase the excretion of alpha-methyl-*m*-tyramine during the first 4 hr (Table 2, Fig. 2B). Between 4 and 12 hr the cold-stressed rats excreted significantly less ($P<0.05$) alpha-methyl-*m*-tyramine than rats placed at 27° C.

Alpha-methyl-*m*-tyrosine depressed heart noradrenaline stores at both ambient temperatures (Table 1, Figs. 3A and B). Hearts removed from the rats placed at 4° C contained significantly more noradrenaline ($P<0.05$) than did hearts taken from rats at 27° C.

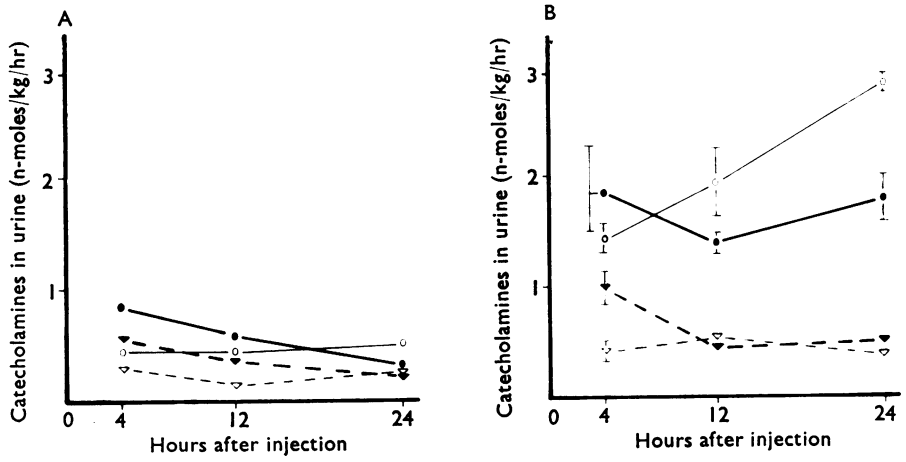

FIG. 4. Urinary excretion of noradrenaline (●—● treated rats, ○—○ control rats) and adrenaline (▼—▼ treated rats, ▽—▽ control rats) from rats kept at 27° C (A) or 4° C (B). Points are means of eight to sixteen rats. Standard errors are plotted for some of the mean values of 4° C.

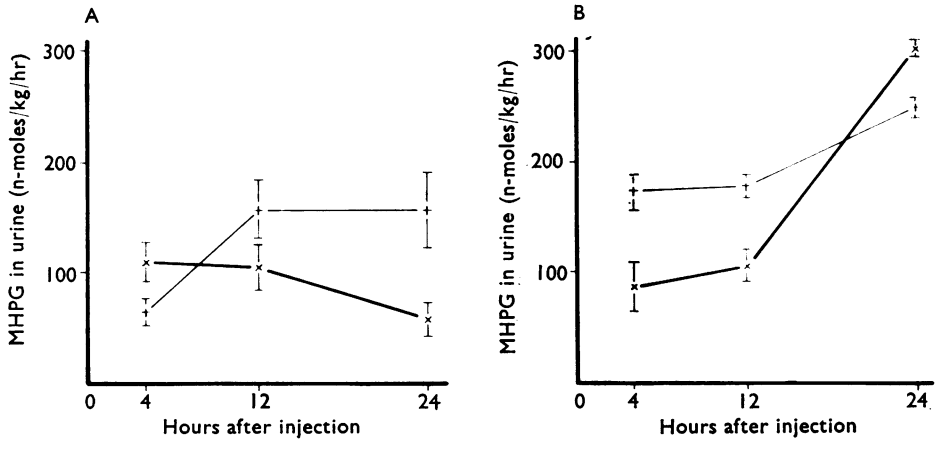

FIG. 5. Urinary excretion of 3-methoxy-4-hydroxy-phenylglycol from control (+—+) and treated (×—×) rats kept at 27° C (A) or 4° C (B). Points are means of eight to sixteen rats. Standard errors are plotted for each mean value.

Treatment of the rats at 27° C with alpha-methyl-*m*-tyrosine produced an initial (0–4 hr) small increase in noradrenaline excretion (Table 2, Fig. 4). Thereafter, the concentration of noradrenaline in the urine fell steadily. Both drug and vehicle-treated rats placed at 4° C excreted large quantities of noradrenaline within the first 4 hr. Thereafter, the level of noradrenaline in the vehicle-treated animals continued to rise, exceeding the quantity excreted in the drug-treated rats between 12 and 24 hr ($P<0.05$). Alpha-methyl-*m*-tyrosine significantly increased the excretion of adrenaline in the cold-stressed rats during hours 0 to 4 ($P<0.05$).

Treatment of rats at 27° C with alpha-methyl-*m*-tyrosine caused an initial non-significant increase in MHPG excretion. This was followed by a subsequent fall with the treated animals excreting significantly less MHPG ($P<0.05$) than controls during hours 12 to 24 (Table 2, Fig. 5). At 4° C, alpha-methyl-*m*-tyrosine significantly depressed the excretion of MHPG during the first 12 hr after treatment; thereafter, the excretion rose and exceeded the amount in the urine of controls receiving only vehicle.

Discussion

The results show that sympathetic stimulation, produced by exposure to cold, increases both the formation and release of metaraminol. Four hours after treatment with alpha-methyl-*m*-tyrosine, hearts removed from rats exposed to 4° C contained significantly more alpha-methyl-*m*-tyramine and metaraminol than hearts taken from animals kept at 27° C. The higher tissue amine levels seen in the cold were accompanied by an increased release, as shown by higher initial excretion of metaraminol in the urine of the animals exposed to cold. Further evidence of the increased release of metaraminol in the cold may be seen in its total depletion from the hearts 12 hr after treatment. This work is consistent with an earlier report by Johnson & Mickle (1966), who demonstrated that exposure to cold increases the release of exogenously supplied metaraminol. It must therefore be concluded that metaraminol satisfies some of the criteria required of a false transmitter in that both its synthesis and release are determined, at least partly, by the state of activity of the sympathetic nervous system.

It may also be inferred from the results that the rate of loss of metaraminol varies from tissue to tissue. The observation that cold-stressed rats continued to excrete small quantities of metaraminol between 12 and 24 hr, at a time when the heart was completely depleted of the amine, indicates the presence of metaraminol in other tissues. This should not be considered unusual, for Gutman & Weil-Malherbe (1967) reported that exposure to cold caused a greater increase in the turnover of ^{3}H-noradrenaline in the heart than in the spleen.

Cold exposure increased the formation of alpha-methyl-*m*-tyramine but did not increase its excretion. This observation may indicate that the 4° C ambient temperature increased the percentage of alpha-methyl-*m*-tyramine subsequently converted into metaraminol.

The displacement of noradrenaline by metaraminol occurred at both 27° C and 4° C, with the effect being greater in the warm room. This agrees with the previous work (Johnson & Mickle, 1966) that cold stress diminishes the catecholamine-depleting action of injected metaraminol. The higher concentrations of noradrenaline in

the heart at 4° C may be a result of a cold-induced increased synthesis of the amine and/or an increased release of metaraminol to allow for catecholamine binding.

Previous workers have suggested that the depression of tissue noradrenaline is dependent on a mole-for-mole replacement by metaraminol (Andén, 1964; Shore *et al.*, 1964). This concept has, however, not been universally accepted (Udenfriend & Zaltzman-Nirenberg, 1964; Johnson & Mickle, 1966; Porter, Torchiana, Totaro & Stone, 1967). The results presented here do not support the mole-for-mole concept. Although an approximate mole-for-mole replacement by metaraminol of noradrenaline was seen at hours 4 and 12 at 27° C, no correlation between metaraminol storage and noradrenaline loss existed at 4° C (Fig. 6). Thus although the loss of noradrenaline may, in certain conditions, be accompanied by an approximate metaraminol replacement, this is not always observed and catecholamine depletion is not dependent on an equimolar uptake of metaraminol.

As expected in view of the loss of noradrenaline from tissue sites, alpha-methyl-*m*-tyrosine increased noradrenaline excretion at 27° C, which after the fourth hour fell to control levels. Alpha-methyl-*m*-tyrosine depressed noradrenaline excretion in the cold stressed rats, with the difference between the control and treated animals being significant between 12 and 24 hr. In spite of the decreased excretion of noradrenaline, however, all treated rats remained normothermic throughout the 24 hr study. Previous workers have emphasized the importance of an increased

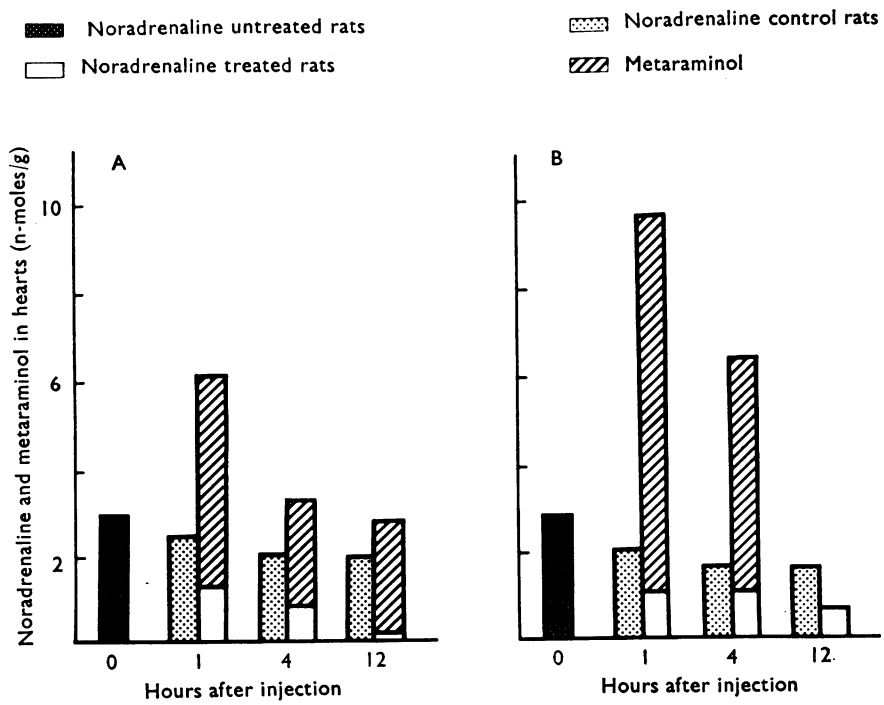

FIG. 6. Concentrations of noradrenaline and metaraminol in the hearts of untreated rats or rats injected either with the vehicle or alpha-methyl-*m*-tyrosine and kept at 27° C (A) or 4° C (B).

release of noradrenaline to the maintenance of normothermia in a cold environment (Leduc, 1961 ; Johnson & Pritzker, 1966, and others). It is possible, in our experiment, that although noradrenaline excretion was below control levels the quantities released were adequate to meet the needs of the animals. It is also likely that other mechanisms of producing heat—for example, shivering—assisted in the maintenance of body temperature. An earlier publication (Johnson & Mickle, 1966) showed that the injection of metaraminol failed to increase the oxygen consumption of rats. It is therefore unlikely that the release of metaraminol substituted in part for the thermogenic actions of noradrenaline.

3-Methoxy-4-hydroxyphenylglycol is a deaminated, O-methylated metabolite of both noradrenaline and adrenaline. Evidence exists to suggest that the major pathway for its formation is first oxidative-deamination, accomplished intracellularly by monoamine oxidase before secretion from the nerve or chromaffin cell, followed by O-methylation after the release of the deaminated metabolite from the nerve or chromaffin cell (Kopin, Hertting & Gordon, 1962 ; Wurtman, 1965). If most of the MHPG found in urine represents catecholamine synthesis and degradation before secretion, its concentration does not reflect a level of active amine secreted. The urinary concentrations of the compound are, however, approximately 100 times the amounts of adrenaline and noradrenaline measured, and therefore the quantity of MHPG excreted may be taken as an index of catecholamine (chiefly noradrenaline) synthesis. As indicated by these values, alpha-methyl-*m*-tyrosine decreased noradrenaline synthesis. Previous studies by Spector, Gordon, Sjoerdsma & Udenfriend (1967) demonstrated that high tissue concentrations of noradrenaline depressed subsequent catecholamine synthesis. It has been postulated that high concentrations of noradrenaline or adrenaline inhibit tyrosine hydroxylase and by end-product inhibition decrease further catecholamine synthesis (Gordon *et al.*, 1966 ; Spector *et al.*, 1967). Whether these suggested mechanisms hold true for metaraminol is not known. The parallel effects of both noradrenaline and metaraminol on catecholamine synthesis and the observation that depletion of tissue metaraminol in the cold coincided with an increase in MHPG, however, provides additional support for the false transmitter concept. It is also possible that the reduction in levels of this material does not reflect a decrease in catecholamine synthesis. Its formation is dependent on the activity of both monoamine oxidase and catechol-O-methyl transferase, and a depression of either of these enzymes following the injection of alpha-methyl-*m*-tyrosine would result in a fall in excretion of the glycol. Although, to our knowledge, no evidence exists to suggest such an inhibition after alpha-methyl-*m*-tyrosine treatment, the possibility of this occurring and influencing the above discussion must be kept open.

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