Br. J. Pharmac. (1970), **39**, 167–174.

Studies on the interrelationship between the syntheses of noradrenaline and metaraminol

G. E. JOHNSON AND T. A. PUGSLEY

Department of Pharmacology, University of Toronto, Toronto 5, Canada

Summary

1. Experiments were conducted to determine the influence of the rate of noradrenaline synthesis on the conversion of alpha-methyl-*m*-tyrosine to metaraminol.

2. Male Wistar rats, 175–200 g, were placed into four groups and treated with (1) alpha-methyl-*p*-tyrosine methyl ester, 250 mg/kg ; (2) DL-alpha-methyl-*m*-tyrosine, 400 mg/kg ; (3) alpha-methyl-*p*-tyrosine methyl ester, 250 mg/kg plus DL-alpha-methyl-*m*-tyrosine, 400 mg/kg ; or (4) an equivalent volume of injection vehicle. All solutions were injected intraperitoneally.

3. Immediately after treatment half of the rats were transferred to 4° C with the remaining animals being kept at 27° C.

4. The rats were killed 4, 8 and 12 h after injection, the brains, hearts, spleens and adrenals removed and analysed for adrenaline, noradrenaline, metaraminol and alpha-methyl-*m*-tyramine.

5. In virtually all cases, both during rest (27° C) and sympathetic stress (4° C), treatment of the rats with alpha-methyl-*p*-tyrosine methyl ester increased the amount of metaraminol formed from alpha-methyl-*m*-tyrosine. The only organ not containing increased quantities of metaraminol in the presence of alpha-methyl-*p*-tyrosine methyl ester was the adrenals, taken from the rats kept at 27° C. Adrenals removed from the cold-exposed rats contained more metaraminol when alpha-methyl-*p*-tyrosine methyl ester was combined with alpha-methyl-*m*-tyrosine than when alpha-methyl-*m*-tyrosine was used alone.

6. These results demonstrate that the inhibition of noradrenaline synthesis, by treatment with the tyrosine hydroxylase inhibitor alpha-methyl-*p*-tyrosine methyl ester, increased the conversion of alpha-methyl-*m*-tyrosine to metaraminol. It is concluded that inhibiting the formation of dopa allowed increased amounts of alpha-methyl-*m*-tyrosine to enter the biosynthetic pathway. These results support the false sympathetic transmitter concept advanced for metaraminol.

Introduction

Metaraminol accumulates in sympathetic nerves replacing, to a large extent, the endogenous noradrenaline (Shore, Busfield & Alpers, 1964 ; Andén, 1964). Metaraminol may be formed from administered alpha-methyl-*m*-tyrosine within sympathetic nerves (Carlsson & Lindqvist, 1962) and its synthesis is increased by

sympathetic stimulation (Johnson & Pugsley, 1968). Once bound within the nerves, metaraminol may be released by catecholamine releasing drugs or by *in vitro* (Crout, Alpers, Tatum & Shore, 1964) or *in vivo* sympathetic stimulation (Johnson & Mickle, 1966 ; Costa, Neff & Ngai, 1969). On the basis of this evidence it has been postulated that metaraminol may function as a false sympathetic transmitter.

During the past few years experiments have been conducted in our laboratory to study various aspects of the false transmitter theory. Our results have been published in a series of articles (Johnson & Mickle, 1966 ; Johnson & Pugsley, 1968). The present paper is a continuation of this programme. Both noradrenaline and metaraminol share the same biosynthetic pathway. Because of this, a study was undertaken to determine the influence of the rate of noradrenaline synthesis on the conversion of alpha-methyl-*m*-tyrosine to metaraminol.

Cold exposure increases the rate of synthesis of both metaraminol (Johnson & Pugsley, 1968) and noradrenaline (Oliverio & Stjärne, 1965). Use was made of this fact to study the interaction between noradrenaline and metaraminol synthesis under conditions of sympathetic rest, 27° C, and stress, 4° C.

Methods

Male Wistar rats, 175–200 g, obtained from the High Oaks Breeding Laboratories, were used throughout the study. The rats were divided into four groups and kept at 27° ± 1° C for at least 24 h before the experiment. The groups were injected intraperitoneally as follows: group 1, alpha-methyl-*p*-tyrosine methyl ester, 250 mg/kg ; group 2, DL-alpha-methyl-*m*-tyrosine, 400 mg/kg ; group 3, alpha-methyl-*p*-tyrosine methyl ester, 250 mg/kg plus DL-alpha-methyl-*m*-tyrosine, 400 mg/kg ; group 4, an equivalent volume of injection vehicle. Immediately after treatment half of the rats were transferred to 4° C and the remaining animals were kept at 27° C.

The animals were placed in individual stainless steel cages. Four, eight and twelve hours after injection the rats were killed and the brains, hearts, spleens and adrenals quickly removed. These tissues were homogenized in 0·4 N perchloric acid and analysed for adrenaline, noradrenaline (Euler & Lishajko, 1961), metaraminol and alpha-methyl-*m*-tyramine (Pugsley & Johnson, 1968).

Results

The results of the tissue analyses are given in Tables 1, 2, 3 and 4 and Fig. 1. Alpha-methyl-*p*-tyrosine methyl ester produced a marked fall in the noradrenaline levels in the hearts and brains with the initial effect being greater in the cold-exposed rats ($P<0{\cdot}01$). The drug caused a smaller fall in the noradrenaline stores in the spleen. The catecholamine levels in the adrenals fell only slightly at 27° C. At 4° C the effect of alpha-methyl-*p*-tyrosine methyl ester on the adrenals was greater.

Alpha-methyl-*m*-tyrosine lowered the tissue levels of noradrenaline in the hearts, spleens and brains ($P<0{\cdot}01$ at 4 h). The catecholamine depleting action of the drug was greater than that of the ester of the *para* isomer. The catecholamine stores in the adrenals fell slightly. Accompanying the fall in noradrenaline, the administration of alpha-methyl-*m*-tyrosine produced high levels of metaraminol and alpha-methyl-*m*-tyramine in the hearts, spleens and brains. The initial

TABLE 1. *Concentrations of noradrenaline (NA), metaraminol (MA) and alpha-methyl-*m*-tyramine (MMTA) in brains of control rats and those treated with alpha-methyl-*p*-tyrosine (MPT, 250 mg/kg), alpha-methyl-*m*-tyrosine (MMT, 400 mg/kg) or a combination of alpha-methyl-*p*-tyrosine (250 mg/kg) and alpha-methyl-*m*-tyrosine (400 mg/kg) kept at 27° C and 4° C*

		Control	MPT	MMT			MMT+MPT		
Temp.	Hours	NA	NA	NA	MA	MMTA	NA	MA	MMTA
27° C	0	0·79±0·03							
	4	0·81±0·01	0·30±0·015	0·067±0·014	0·95±0·014	2·32±0·238	0	1·27±0·09†	2·08±0·17
	8	0·71±0·01	0·51±0·004	0·06±0·006	1·33±0·017	0·19±0·006	0·067±0·004	2·34±0·02*	2·02±0·003
	12	0·63±0·01	0·25±0·014	0·047±0·008	1·15±0·019	0·73±0·16	0·114±0·004	3·46±0·07*	1·84±0·07
4° C	0	0·79±0·02							
	4	0·94±0·02	0·17±0·01	0·098±0·008	1·97±0·07	2·22±0·10	0·003±0·0016	1·48±0·03	3·59±0·38
	8	1·10±0·04	0·12±0·007	0·19±0·004	1·13±0·09	1·01±0·13	0·14±0·004	1·94±0·09*	0·76±0·08
	12	0·88±0·06	0·60±0·02	0·17±0·008	1·63±0·03	0·70±0·17	0·108±0·012	3·31±0·06*	1·06±0·095

Values in the table are expressed as nmol/g of fresh tissue. The time intervals indicate the time of death after injection. Values given represent the means of four samples ± standard error. The metaraminol and alpha-methyl-*m*-tyramine values have been corrected for incomplete recovery and tissue blanks.

* $P<0·01$ as compared with rats treated with only alpha-methyl-*m*-tyrosine.
† $P<0·05$.

TABLE 2. *Concentrations of noradrenaline (NA), metaraminol (MA), and alpha-methyl-*m*-tyramine (MMTA) in hearts of control rats and those treated with alpha-methyl-*p*-tyrosine (MPT, 250 mg/kg), alpha-methyl-*m*-tyrosine (MMT, 400 mg/kg) or a combination of alpha-methyl-*p*-tyrosine (250 mg/kg) and alpha-methyl-*m*-tyrosine (400 mg/kg) kept at 27° C and 4° C*

		Control	MPT	MMT			MMT+MPT		
Temp.	Hours	NA	NA	NA	MA	MMTA	NA	MA	MMTA
27° C	0	2·06±0·145							
	4	2·10±0·14	1·14±0·083	0·41±0·02	2·01±0·08	2·91±0·11	0·08±0·01	1·35±0·03†	0·94±0·05
	8	2·05±0·21	0	0·26±0·016	2·01±0·07	4·98±0·07	0·05±0·02	4·20±0·04*	2·28±0·18
	12	2·00±0·13	0·66±0·031	0·11±0·01	2·66±0·10	1·43±0·05	0·08±0·01	3·93±0·07*	3·52±0·41
4° C	0	2·06±0·145							
	4	1·24±0·12	0·38±0·02	0·40±0·04	2·99±0·09	3·21±0·54	0	3·70±0·18†	1·64±0·14
	8	1·17±0·07	0·44±0·015	0·17±0·021	1·53±0·02	0·66±0·04	0	2·99±0·09*	0·88±0·30
	12	1·21±0·095	0·50±0·02	0·33±0·02	1·05±0·02	0	0·52±0·056	4·54±0·10*	4·79±0·08

Values in the table are expressed as nmol/g of fresh tissue. The time intervals indicate the time of death after injection. Values given represent the means of four samples ±standard error. The metaraminol and alpha-methyl-*m*-tyramine values have been corrected for incomplete recovery and tissue blanks.

* $P<0·01$
† $P<0·05$ } as compared with rats treated with only alpha-methyl-*m*-tyrosine.

TABLE 3. *Concentrations of noradrenaline (NA), metaraminol (MA), and alpha-methyl-*m*-tyramine (MMTA) in spleens of control rats and those treated with alpha-methyl-*p*-tyrosine (MPT, 250 mg/kg), alpha-methyl-*m*-tyrosine (MMT, 400 mg/kg) or a combination of alpha-methyl-*p*-tyrosine (250 mg/kg) and alpha-methyl-*m*-tyrosine (400 mg/kg) kept at 27° C and 4° C*

		Control	MPT	MMT			MMT+MPT		
Temp.	Hours	NA	NA	NA	MA	MMTA	NA	MA	MMTA
27° C	0	1·57±0·02							
	4	1·47±0·02	1·15±0·03	0·49±0·05	3·10±0·02	2·20±0·03	0·37±0·02	6·11±0·61*	4·15±0·03
	8	1·52±0·02	0·97±0·02	0·25±0·008	3·80±0·17	0·50±0·03	0·55±0·012	5·96±0·11*	1·52±0·02
	12	1·65±0·03	0·66±0·07	0·21±0·017	1·70±0·06	0·30±0·06	0·08±0·025	3·14±0·34*	2·96±0·17
4° C	0	1·57±0·02							
	4	1·44±0·05	1·06±0·03	0·18±0·016	2·08±0·13	7·75±0·18	0·75±0·02	10·47±0·79*	9·38±1·16
	8	1·50±0·09	0·53±0·02	0	3·74±0·12	2·57±0·06	0·095±0·013	10·82±0·89*	3·94±0·05
	12	1·68±0·09	0·65±0·01	0·88±0·01	12·35±0·23	5·97±0·20	0·46±0·021	10·12±0·30	3·54±0·12

Values in the table are expressed as nmol/g of fresh tissue. The time intervals indicate the time of death after injection. Values given represent the means of four samples±standard error. The metaraminol and alpha-methyl-*m*-tyramine values have been corrected for incomplete recovery and tissue blanks.

*$P<0·01$ as compared with rats treated with only alpha-*m*-tyrosine.

TABLE 4. *Concentrations of noradrenaline (NA), adrenaline (A), metaraminol (MA), and alpha-methyl-*m*-tyramine (MMTA) in adrenals of control rats and those treated with alpha-methyl-*p*-tyrosine (MPT, 250 mg/kg), alpha-methyl-*m*-tyrosine (MMT, 400 mg/kg) or a combination of alpha-methyl-*p*-tyrosine (250 mg/kg) and alpha-methyl-*m*-tyrosine (400 mg/kg) kept at 27° C and 4° C*

		Control		MPT		MMT				MMT+MPT			
Temp.	Hours	NA	A	NA	A	NA	A	MA	MMTA	NA	A	MA	MMTA
27° C	0	72·22 ±3·51	459·50 ±14·41										
	4	75·71 ±9·63	412·34 ±8·52	80·50 ±4·73	399·18 ±5·19	97·28 ±5·79	283·62 ±4·80	7·10 ±0·50	0	95·39 ±4·67	346·34 ±3·71	6·15 ±0·47	6·62 ±0·72
	8	71·31 ±3·84	428·71 ±3·60	41·90 ±5·91	332·42 ±5·08	81·50 ±11·88	341·81 ±3·99	14·66 ±0·42	7·84 ±0·73	107·03 ±4·84	335·64 ±4·51	4·49 ±0·39	10·41 ±0·82
	12	65·60 ±4·17	436·95 ±10·37	105·14 ±5·44	340·45 ±1·80	129·49 ±6·27	418·72 ±5·35	20·21 ±0·72	9·39 ±1·11	125·24 ±3·55	393·56 ±3·82	9·99 ±0·55	7·98 ±0·51
4° C	4	71·28 ±6·32	433·08 ±7·97	45·57 ±5·32	316·32 ±1·91	8·39 ±3·19	308·52 ±4·59	9·02 ±0·31	0	0	353·71 ±4·75	32·31* ±1·20	9·48 ±0·86
	8	86·47 ±2·96	409·50 ±4·37	36·58 ±8·81	236·30 ±11·19	31·56 ±3·66	279·97 ±1·58	9·70 ±0·36	11·75 ±0·39	15·31 ±1·55	326·69 ±6·82	22·47 ±0·42	4·97 ±0·35
	12	107·92 ±3·55	497·22 ±12·28	21·16 ±3·78	335·37 ±2·18	32·51 ±2·54	236·90 ±1·53	15·01 ±0·51	6·43 ±0·86	19·50 ±2·54	338·37 ±2·57	15·45 ±0·58	2·85 ±0·73

Values in the table are expressed as nmol/kg body weight. The time intervals indicate the time of death after injection. Values given represent the means of four samples ± standard error. The metaraminol and alpha-methyl-*m*-tyramine values have been corrected for incomplete recovery and tissue blanks.

* $P<0·01$ as compared with rats treated with only alpha-methyl-*m*-tyrosine.

levels of metaraminol in the brains and hearts were higher in the rats kept at 4° C ($P<0.05$). In the spleen the initial levels were higher in the rats kept at 27° C ($P<0.01$). Twelve hours after treatment spleens removed from the cold-exposed animals contained considerably more metaraminol than did spleens taken from the warm-room rats. Metaraminol and alpha-methyl-*m*-tyramine were also found in the adrenals of both groups of rats.

Treatment of the rats with alpha-methyl-*p*-tyrosine methyl ester increased the amount of metaraminol formed from alpha-methyl-*m*-tyrosine. On occasion the increased formation of metaraminol by rats given both drugs was not immediately seen; for example brain 4 h sample at 4° C and heart 4 h sample at 27° C. However, when these tissues were removed either 8 or 12 h after injection, the potentiating action of alpha-methyl-*p*-tyrosine methyl ester was seen. The only organ not containing increased quantities of metaraminol in the presence of alpha-methyl-*p*-tyrosine methyl ester was the adrenals. Adrenals removed from the rats kept at 27° C contained less metaraminol when alpha-methyl-*m*-tyrosine was combined with alpha-methyl-*p*-tyrosine methyl ester than when alpha-methyl-*m*-tyrosine was given alone. Treatment of the cold-exposed rats with both drugs increased the amount of metaraminol found in the adrenals.

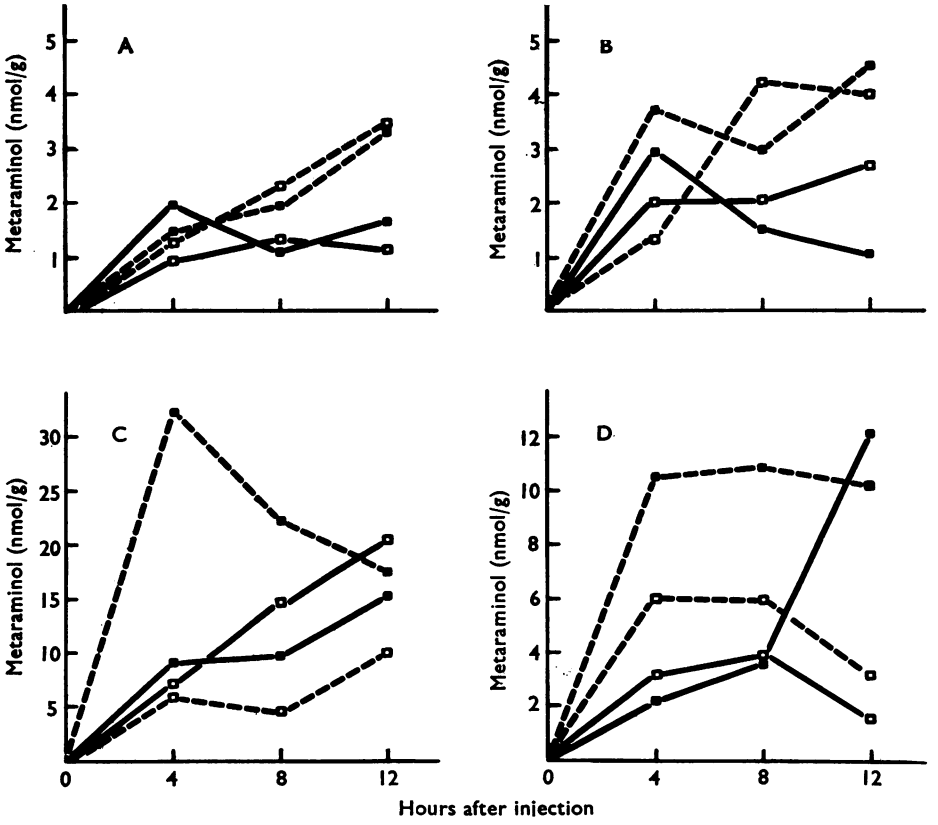

FIG. 1. Concentrations of metaraminol in brains (A), hearts (B), adrenals (C) and spleens (D) of rats after the administration of alpha-methyl-*m*-tyrosine (——), and alpha-methyl-*m*-tyrosine plus alpha-methyl-*p*-tyrosine (- - - -), at 27° C (□) or 4° C (■). Plotted values are means of four determinations.

Discussion

The concept of metaraminol as a false sympathetic transmitter is based on the evidence that it replaces noradrenaline within sympathetic nerves and is subject to release by sympathetic stimulation (Crout *et al.*, 1964 ; Johnson & Mickle, 1966). Metaraminol may be synthesized within sympathetic nerves from previously administered alpha-methyl-*m*-tyrosine (Carlsson & Lindqvist, 1962). Evidence of this was seen in our study. Following the injection of alpha-methyl-*m*-tyrosine, all tissues analysed contained high concentrations of both alpha-methyl-*m*-tyramine and metaraminol. Noradrenaline stores fell as the catecholamine was replaced by metaraminol. Considerable work has been published on various aspects of the interactions between noradrenaline and metaraminol (see Crout (1966) and Kopin (1968a, b) for references). However, in spite of the fact that the synthesis of metaraminol in sympathetic nerves utilizes two enzymes normally employed in noradrenaline formation—namely, L-aromatic amino-acid decarboxylase and dopamine-β-oxidase—no work has appeared describing the influence of the rate of noradrenaline synthesis on the conversion of alpha-methyl-*m*-tyrosine to metaraminol.

The chemical alpha-methyl-*p*-tyrosine methyl ester is an effective inhibitor of tyrosine hydroxylase and has been used by numerous workers to inhibit noradrenaline synthesis. Alpha-methyl-*p*-tyrosine methyl ester was used as a tool to block noradrenaline synthesis at a stage, the conversion of tyrosine to dopa, that would not impair the formation of metaraminol from alpha-methyl-*m*-tyrosine. The dose used in the present experiments, 250 mg/kg, was selected on the basis of studies by Corrodi & Hansson (1966) and Corrodi & Malmfors (1966), who showed an inhibition of tyrosine hydroxylase produced a fall in noradrenaline and dopamine tissue concentrations. Evidence of the effects of the alpha-methyl-*p*-tyrosine methyl ester in our study can be seen by the fall in noradrenaline tissue levels after its injection. The effect was more pronounced at 4° C, due to the increased turnover of noradrenaline in the cold.

The inhibition of noradrenaline synthesis increased the formation of metaraminol from alpha-methyl-*m*-tyrosine. Brains, heart and spleens taken from rats given both alpha-methyl-*m*-tyrosine and alpha-methyl-*p*-tyrosine methyl ester contained, in almost all cases, more metaraminol than the corresponding organs removed from the animals given only alpha-methyl-*m*-tyrosine. Spector, Sjoerdsma & Udenfriend (1965) previously reported that alpha-methyl-*p*-tyrosine increased the incorporation of dopa-^{3}H into tissue catecholamines. These workers concluded that a decrease in the formation of endogenous dopa allowed the entry of the labelled analogue into the pathway with less dilution. Our results suggest that inhibiting the formation of dopa allowed increased amounts of alpha-methyl-*m*-tyrosine to enter the biosynthetic pathway. This information demonstrates a competition between the synthesis of noradrenaline and metaraminol and reinforces the false transmitter concept.

Cold exposure increases noradrenaline turnover (Oliverio & Stjärne, 1965 ; Costa, Neff & Ngai, 1969) and can, following the administration of alpha-methyl-*m*-tyrosine, increase the formation of metaraminol (Johnson & Pugsley, 1968). Four hours after treatment with alpha-methyl-*m*-tyrosine higher levels of metaraminol were found in the hearts and brains of the cold-exposed rats. The increased rate

of metaraminol formation, seen at 4° C, was further elevated by alpha-methyl-*p*-tyrosine treatment. It can be concluded that, under conditions of both sympathetic rest or stress, the rate of metaraminol formation can be influenced by the amount of tyrosine being converted to dopa.

Inhibition of tyrosine hydroxylase did not increase the formation of metaraminol in the adrenals of the rats kept at 27° C. In fact, in this gland treatment with alpha-methyl-*m*-tyrosine alone produced higher levels of metaraminol than alpha-methyl-*m*-tyrosine and alpha-methyl-*p*-tyrosine combined. In the warm the turnover of catecholamines in the adrenals is depressed and this probably accounts for the failure of tyrosine hydroxylase inhibition to increase metaraminol formation. In the cold, under conditions of sympathetic stress and increased catecholamine turnover, alpha-methyl-*p*-tyrosine increased the formation of metaraminol in the adrenals. Although significant amounts of metaraminol were found in the adrenals, they represented only a small fraction of the normal catecholamine levels. The drug produced only a small fall in the normal stores of adrenaline and noradrenaline.

One additional point deserves comment. Cold exposure increased the formation of metaraminol in hearts and brains, with the increase being noted 4 h after injection. Cold exposure did not increase the formation of metaraminol by the spleen during the first 8 h after treatment. It has been a consistent observation in our laboratory that the release of noradrenaline in the spleen is relatively refractive to cold exposure. Because of this it is not surprising to observe that cold exposure produced no immediate increase in metaraminol synthesis. Twelve hours after treatment the spleens removed from the cold-stressed rats contained many times the levels of metaraminol found in the animals kept at 27° C.

This work was supported by grant 1595 from the Medical Research Council of Canada. T. A. Pugsley is a recipient of a Medical Research Council of Canada Studentship. Alpha-methyl-*p*-tyrosine methyl ester (H 44/68) was kindly supplied by Dr. H. Corrodi, Hässle AB, Gothenburg, Sweden.

REFERENCES

ANDÉN, N. E. (1964). On the mechanism of noradrenaline depletion by alpha-methyl-*m*-tyrosine and metaraminol. *Acta pharmac. tox.*, **31**, 260–271.

CARLSSON, A. & LINDQVIST, M. (1962). In vivo decarboxylation of alpha-methyl-dopa and alpha-methyl-meta-tyrosine. *Acta physiol. scand.*, **54**, 87–94.

COSTA, E., NEFF, N. H. & NGAI, S. H. (1969). Regulation of metaraminol efflux from rat heart and salivary gland. *Br. J. Pharmac. Chemother.*, **28**, 153–160.

CORRODI, H. & HANSSON, K. (1966). Central effects of an inhibitor of tyrosine hydroxylation. *Psychopharmacology*, **10**, 116–125.

CORRODI, H. & MALMFORS, T. (1966). The effect of nerve activity on the depletion of the adrenergic transmitter by inhibitors of noradrenaline synthesis. *Acta physiol. scand.*, **67**, 352–357.

CROUT, J. R., ALPERS, H. S., TATUM, E. L. & SHORE, P. A. (1964). Release of metaraminol (aramine) from the heart by sympathetic nerve stimulation. *Science, N.Y.*, **145**, 828–829.

CROUT, J. R. (1966). Substitute adrenergic transmitters. *Circulation Res.*, **18**, Suppl. 1, 120–127.

EULER, U. S. v. & LISHAJKO, F. (1961). Improved technique for the fluorometric estimation of catecholamines. *Acta physiol. scand.*, **51**, 348–355.

JOHNSON, G. E. & MICKLE, D. (1966). The influence of cold exposure on the *in vivo* release of metaraminol. *Br. J. Pharmac. Chemother.*, **28**, 246–254.

JOHNSON, G. E. & PUGSLEY, T. A. (1968). The formation and release of metaraminol during exposure to warm and cold environments. *Br. J. Pharmac.*, **34**, 267–276.

KOPIN, I. J. (1968a). False adrenergic transmitters. *Ann. Rev. Pharmac.*, **8**, 377–394.

KOPIN, I. J. (1968b). The influence of false adrenergic transmitters on adrenergic transmission. *Adrenergic Neurotransmission*, ed. Wolstenholme, G. E. W. & O'Connor, M., pp. 95–104. London: J. A. Churchill.

OLIVERIO, A. & STJÄRNE, L. (1965). Acceleration of noradrenaline turnover in mouse heart by cold exposure. *Life Sci., Oxford*, **4**, 2330–2343.

PUGSLEY, T. A. & JOHNSON, G. E. (1968). Modified method for the estimation of metaraminol and alpha-methyl-m-tyramine. *J. Pharm. Pharmac.*, **20**, 490–491.

SHORE, P. A., BUSFIELD, D. & ALPERS, H. S. (1964). Binding and release of metaraminol: mechanism of norepinephrine depletion by alpha-methyl-m-tyrosine and related agents. *J. Pharmac. exp. Ther.*, **146**, 194–199.

SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Blockade of endogenous norepinephrine synthesis by alpha-methyltyrosine and inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **147**, 86–95.

(*Received January* 9, 1970)