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DEMONSTRATION OF METARAMINOL IN NERVOUS AND NON-NERVOUS TISSUES BY A HISTOCHEMICAL FLUORESCENCE REACTION

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Abstract—The metaraminol present extracellularly in brain and tongue tissues and that taken up intracellularly by heart muscle fibres and certain gland cells following respectively the local injection and systemic administration of the amine is, just as in models, readily converted to a strongly fluorescent product on treatment of the tissues according to the histochemical fluorescence method of Falck and Hillarp for the cellular localization of catecholamines. However, this conversion to a fluorescent product does not take place to any significant extent, when metaraminol or closely related metatyramines are present in the adrenergic neurons (cell bodies, axons, terminals). The results of the present studies exclude the possibility of this being solely due to inadequate amine concentrations or to a binding to the storage granules different from that of endogenous noradrenaline Certain possible explanations are presented.

ADMINISTRATION of the 3,4-dihydroxyphenylalanine (DOPA) analogues metatyrosine, α-methyl metatyrosine (α-MMT) and α-methyl DOPA produce a depletion of the noradrenaline (NA) stored in both the peripheral adrenergic nerves and the brain.[1, 2] This depletion is brought about by the amines formed intraneuronally through decarboxylation of these analogues. The endogenous NA in the storage granules is thereby displaced by approximately equivalent amounts of these metatyramines or in the case of α-MMT and α-methyl DOPA by the β-hydroxylated derivatives metaraminol and α-methyl NA, which persist for a long time[1, 3, 5].

Both the adrenergic transmitter and the central catecholamines (CA) can be demonstrated directly within the neuron by use of the sensitive histochemical fluorescence method of Falck and Hillarp[6–10]. In the present studies it was found that neither the peripheral adrenergic neuron nor the central CA neurons continued to exhibit fluorescence after a depletion of the endogenous amines by the adminstration of metatyrosine or α-MMT. There was no immediate explanation for this finding, since both the metatyramines and the primary CA are readily converted to intensely fluorescent products by the histochemical treatment (see Discussion). Since the DOPA analogues are useful tools for the study of adrenergic transmission and the metabolism of neuronal CA, more detailed investigation of this problem has been made in the present paper.

MATERIAL AND METHODS

Some 200 Sprague-Dawley rats with a body wt. of about 200 g were used. The adrenergic nerves of the iris were examined after treatment of the animals as shown in Tables 1 and 2. The rats were killed by decapitation under light ether anaesthesia. The irises were immediately prepared as stretch-preparations mounted whole on

microscope slides, dried for about 1 hr over phosphorous pentoxide and treated with gaseous formaldehyde at +80° for 1 hr in a closed vessel containing paraformaldehyde of optimum water content[11]. The techniques used for studies on the iris nerves are described in detail by Malmfors (1965)[12]. Irises from untreated animals always were used as controls to provide a check on the histochemical reaction. Whenever the conversion of the endogenous NA in the adrenergic nerves into its fluorescent product was unsatisfactory in the controls, the irises of the experimental animals were excluded from the study.

TABLE 1. EFFECT OF DRUGS ON FLUORESCENCE INTENSITY OF ADRENERGIC IRIS NERVES

Treatment	Number of animals	Dose mg/kg	Time after last injection (Hours)	Fluorescence intensity
1. Untreated controls	>40			+++
2. α-MMT	4	2×400* i.p.	1–3	(+)–0
	2	,, ,,	24	(+)
	2	,, ,,	48	+
3. α-MMT	4	20 i.p.	8	++
	8	40 ,,	,,	+
	6	80–100 i.p.	,,	+–(+)
	5	400 ,,	,,	(+)
	8	400 s.c.	,,	(+)
4. Metaraminol	2	12 s.c.	2	++
	3	,, ,,	4	+
	3	,, ,,	6	+–(+)
	6	,, ,,	8–24	+–(+)
5. Metaraminol	30	2×12† s.c.	6–9	(+)–0
	4	,, ,,	14	(+)
6. α-Methyl DOPA	10	400 s.c.	8	++
	10	,, ,,	24	++

* 6 hr interval between doses
† 2 hr interval between doses

TABLE 2. EFFECT OF DRUGS ON FLUORESCENCE INTENSITY OF ADRENERGIC IRIS NERVES
(ANIMALS PRETREATED WITH RESERPINE 5 MG/KG I.P. 18–20 HR BEFORE THE EXPERIMENT)

Treatment	Number of animals	Dose mg/kg	Time after last injection (Hours)	Fluorescence intensity
1. Controls, untreated	>15			++++
2. Controls reserpine	10	5 i.p.	20–24	0
3. Metatyrosine*	2	10 s.c.	1	0
	2	50 ,,	,,	0
	2	400 ,,	,,	0
4. Metatryramine*	2	10 i.v.	1/4	0
	2	10 ,,	1	0
5. Metaraminol	4	12 s.c.	1/2–1	0
	7	12 ,,	2–6	0
	2	12 ,,*	1	(+)
	2	2×12† s.c.	6	0

* Pretreated with nialamide (100 mg/kg i.p., 4 hours)
† Two hour interval between doses

In another group of studies, about 5 min after the intraperitoneal administration of urethane (1 g/kg), about 1 µl of a metaraminol solution (0·1 or 1 mg/ml in 0·9% NaCl) was injected with a fine capillary pipette into the tongue of 5 rats and approximately 2 mm below the surface of the parietal lobe in the brain of another 5 rats. The tissue surrounding the deposited solution was removed after 1 or 5 min (tongue and brain respectively) and immediately frozen in propane, cooled by liquid nitrogen. The pieces were freeze-dried, treated with formaldehyde for the histochemical demonstration of primary CA, embedded in paraffin and sectioned (7 to 10µ). The sections were then examined in a fluorescent microscope as described in detail by Dahlström and Fuxe[9].

In 4 additional rats metaraminol (10 mg/kg) was injected intravenously over a 5 min period during urethane anaesthesia. The animals and untreated controls were then immediately killed and pieces of the submandibular glands, vas deferens, heart and tongue were studied in the usual manner after freeze-drying.

The following drugs were used: DL-metatyrosine (Sigma Chemical Co.), DL-α-methyl metatyrosine monohydrate (Regis Chemical Co.), metatyramine hydrochloride (Hoffman La Roche), L-α-methyl DOPA (Aldomet®; Merck Sharp and Dohme), (-)-metaraminol bitartrate (Aramine®; Merck Sharp and Dohme), reserpine (Serpasil; Ciba), and nialamide (Niamide®; Pfizer). The doses of metatyramine and metaraminol were calculated as free base.

RESULTS AND DISCUSSION

When primary CA (or DOPA) enclosed in a dried protein layer are treated with gaseous formaldehyde they are readily converted to intensely fluorescent 6,7-dihydroxy-3,4-dihydroisoquinolines in a proteinpromoted reaction[13, 14]. The same conversion takes place in freeze- or air-dried tissues. It has been shown that the corresponding metahydroxy compounds (metatyrosine, α-MMT, metatyramine and metaraminol) react in a similar way in dried proteinfilm models (Corrodi and Jonsson, unpublished results). The products formed, in all probability 6-hydroxy-3,4-dihydroisoquinolines, show during fluorescent microscopy a strong but more yellowish fluoresence than the products from the CA moreover, the peak of emission differs being about 510 for analogues and 480 mµ for the CA.

In the present study it was found that metaraminol directly injected into the tongue or brain *in vivo* developed a green-yellow fluorescence following the histochemical treatment. The tissues were frozen so quickly after the injections (1 and 5 min, respectively) that there is no reason to suspect that the fluorescence might be due to any metabolite of the metaraminol. An extremely strong fluorescence was obtained in the tongue and slightly less strong fluorescence in the brain tissues, when the metaraminol concentrations in the injected solutions were 1 and 0·1 µg/µl (injected volume: about 1 µl). In the brain, the tissue in a zone about 0·5 mm broad around the injection site showed a diffuse fluorescence only between the nerve cells which failed to fluoresce and appeared as dark areas. The tongue exhibited fluorescence mainly between the muscle fibres and intracellularly in some of the gland ducts. The concentrations in the tissues must be less than can be obtained readily in adrenergic nerves following the administration of α-MMT or metaraminol (see below).

In the animals killed immediately following the slow intravenous injection of a large dose of metaraminol (10 mg/kg), no specific fluorescence—apart from the normal

yellow-green fluorescence due to the presence of NA in the adrenergic terminals—was obtained in the submandibular gland, tongue or vas deferens. The heart, on the other hand, showed a strong green–yellow fluorescence in the muscle fibres while the intermuscular tissue was non-fluorescent, thus indicating that the heart muscle fibres can take up considerable amounts of metaraminol.

Following the administration of large doses of α-MMT or metaraminol, the strong fluorescence of the adrenergic nerve terminals in the iris, (see microphotos[12, 15]), which is due to the presence of NA, disappeared almost completely in about 6 hr (Table 1). The time course and dose dependence agree very well with those of NA depletion as determined chemically[14]. The data in Table 1 agree very well with the data on the NA depletion obtained by chemical analyses[1, 16–19]. Usually, however, a very weak fluorescence persisted, even when two very large doses of metaraminol were given. This fluorescence, probably is the consequence of a small residue of NA, since it is very difficult to deplete the NA stores more than 95 per cent[1, 20]. The rare instances in which no fluorescence was observed could be attributed to a less than perfect histochemical reaction.

After the largest doses of metaraminol or α-MMT and a short duration of the experiments (Table 1) the concentrations of metaraminol and α-methyl-metatyramine present in the terminals very likely were higher than the amounts of displaced NA since the normal concentrations of NA in the varicosities of the terminals is of the order of 10·000 μg/g wet wt[10]. There is also little doubt that the non-terminal axons contained greater or lesser concentrations of the metahydroxy amines in the axoplasma outside the amine storage granules. The latter are very few in number in this part of the adrenergic nerve[12, 21, 22]. The endogenous NA on the other hand, had been replaced by approximately equivalent amounts of metaraminol 24 hr after the administration of α-MMT or metaraminol, and this accumulated metaraminol is apparently bound to the storage granules in the terminals far more firmly than the endogenous NA[1]. In neither case, however, was there observed a fluorescence that could be due to the presence of metaraminol or α-methyl metatyrosine.

The presence of a methyl group in the α position does not seem to influence significantly the development of fluorescence, since α-methyl DOPA and α-methyl NA are as readily converted to intensely fluorescent products as DOPA and NA both in models and tissues including adrenergic nerves[4, 22]. After the administration of a large dose of α-methyl DOPA, the adrenergic terminals in the iris showed no change in fluorescence (Table 1). The same observation has been made in other tissues in rats treated with two doses (400 mg/kg each, at an interval of two hours) and killed 24 hr after the first injection[4]. With this treatment the endogenous NA is displaced in both peripheral and central adrenergic nerves by approximately equivalent amounts of α-methyl NA[2–4, 23].

The experiments thus show that, in contrast to metaraminol, the α-methyl NA formed from α-methyl DOPA in adrenergic terminals and stored in the amine granules develops full fluorescence on the histochemical treatment.

The non-terminal axons, the terminals, and also the cell bodies of peripheral adrenergic neurons have a specific and highly efficient mechanism localized in the cell membrane for the uptake and concentration of NA and related amines (e.g. α-methyl NA)[12, 21].

After the administration of a large dose of α-methyl NA (0·2 mg/kg i.v.), this amine

accumulates in the adrenergic terminals in concentrations comparable to those of endogenous NA. This membrane pump, insensitive to reserpine, in inhibited by desmethylimipramine[12, 24]. That metatyramine and metaraminol are also taken up by this mechanism is supported by data which showed that desmethylimipramine but not reserpine can block their uptake[24], that denervation strongly reduces this uptake (Almgren and Waldeck, unpublished observations) and that even small doses of metaraminol can produce a severe depletion of NA[1,18]. If a large dose of metatyramine or metaraminol is given to reserpinized animals, the amines should accumulate to high levels in the adrenergic nerves, without the accumulated amines being bound to the storage granules since reserpine blocks this uptake-storage mechanism[25, 26]. Experiments performed to determine whether such non-granulebound amines can be converted to fluorescent products are listed in Table 2.

The fluorescence of the iris nerves completely disappeared 18–20 hr after the intraperitoneal administration of reserpine (5 mg/kg). Large doses of metatyrosine or metatyramine were given to such animals, and nialamide was administered 4 hr previously to prevent inactivation of the amines by MAO[12, 21]. In spite of the high doses, no fluorescence reappeared in the nerves. The same occurred after metaraminol, except for 2 rats pretreated with nialamide in which a very weak fluorescence was found which was probably due to very small amounts of NA reappearing as a result of the MAO inhibition[12].

One hour after the highest dose of metatyrosine a marked increase in the diffuse background fluorescence of the iris was observed. This fluorescenee was greenish–yellow and probably due to the presence of a fairly high content of the amino acid in the tissue[7, 9].

The experiments show clearly that the metaraminol present in some tissues can—just as in models—be converted to a product exhibiting a strong greenish–yellow fluorescence by the histochemical method used. They also show, however, that the conversion does not take place to any significant extent when metaraminol or other closely related metahydroxy or α-methyl-metahydroxy amines are present in adrenergic nerves. No adequate explanation has been obtained for this puzzling phenomenon. The results exclude the possibility of this being due solely to low amine concentrations or to a binding to the storage granules being different from that of the endogenous NA. The most likely explanations[13, 14] are: (1) that the intraneuronal proteins are not efficient catalyzers of the reaction for the final step in dehydrogenation, (2) that the environment does not favour the existence of the quinoid form of the 6-hydroxy-3, 4-dihydroiso-quinolines which is in all probability responsible for the fluorescence, or (3) that some intraneuronal constituent effectively quenches or inhibits the histochemical reaction. These possibilities are now under study. It has been observed also in model experiments that the metahydroxy amines sometimes develop no 510 mμ fluorescence but show instead an emission with a peak at 420 mμ (Corrodi and Jonsson, unpublished observations). This may provide a clue as to why the histochemical treatment fails when the amines are present in adrenergic nerves.

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