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Different Acute Effects of the Tyrosine Hydroxylase Inhibitors α-Methyl-p-Tyrosine and 3-Iodo-L-Tyrosine on Hypothalamic Noradrenaline Activity and Adrenocorticotrophin Release in the Rat

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Abstract

Computerized gas chromatography–mass spectrometry techniques using selected ion monitoring and deuterated internal standards were used to assay simultaneously the medial basal hypothalamic concentrations of dopamine (DA) and noradrenaline (NA) and their major metabolites in individual rats 30 min after the administration of two different inhibitors of tyrosine hydroxylase, α-methyl-*p*-tyrosine (α-MT) and 3-iodo-L-tyrosine (MIT). Consistent with inhibition of DA synthesis, administration of both α-MT and MIT resulted in marked reductions ($P<0.005$) in the hypothalamic concentrations of DA and its metabolite homovanillic acid as well as in highly significant increases in prolactin secretion. α-MT administration, but not MIT, resulted in a highly significant decrease in NA concentration and a highly significant increase in the concentration of the NA metabolite 3,4-dihydroxyphenylethyleneglycol (DHPG). The hypothalamic ratio DHPG/NA was thus markedly increased ($P<0.005$) by α-MT indicating increased NA neuronal activity. α-MT administration also resulted in increased ACTH secretion ($P<0.0005$), an effect not observed following MIT.

It is proposed that the effects on hypothalamic NA activity and ACTH secretion caused by α-MT are stress-mediated and unrelated to tyrosine hydroxylase inhibition. MIT is devoid of these effects but exhibits DA synthesis blockade activity, thus indicating it to be a preferable drug for the acute inhibition of tyrosine hydroxylase in neuroendocrine investigations.

Introduction

The use of α-methyl-*p*-tyrosine (α-MT) to inhibit tyrosine hydroxylase (i.e. tyrosine 3-monooxygenase, E.C. 1.14.16.2) activity has been a widely used technique in the investigation of the roles of brain catecholamines in neuroendocrine control mechanisms (Muller *et al.* 1977). Because tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of dopamine (DA) and noradrenaline (NA) from the initial amino acid precursor, L-tyrosine (Nagatsu *et al.* 1964), its specific inhibition can be used as a tool in assessing turnover rates of the catecholamines as well as determining the effects of their depletion in various physiological situations. It has been a common practice in these kinds of investigations to use a drug such as α-MT with a known action on tyrosine hydroxylase, without consideration of any other actions the drug might have, and to ascribe all the consequences of administration of that drug to a single and 'specific' activity. Notwithstanding the fact that only one type of activity might have been demonstrated for a particular drug, other activities might exist and caution is necessary in interpreting drug effects in endocrine studies (Smythe 1977). In the case of the use of α-MT, the overriding assumption has been that this compound is a specific inhibitor of tyrosine hydroxylase (Muller *et al.* 1977) and its effects in stimulating ACTH were of importance in the development of the hypothesis that hypothalamic NA exerted inhibitory control over ACTH release (Muller *et al.* 1977). 3-Iodo-L-tyrosine is another potent inhibitor of tyrosine

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hydroxylase (Udenfriend *et al.* 1965) which we have used to inhibit DA synthesis in several studies (see Smythe *et al.* 1982*a*). While MIT is a more potent tyrosine hydroxylase inhibitor than α-MT (Udenfriend *et al.* 1965), in no investigation using MIT have we been able to observe any acute stimulation of ACTH such as had been shown for α-MT (Scapagnini *et al.* 1970). This apparent anomaly led us to suspect that either MIT or α-MT exerted effects other than that shared in common of tyrosine hydroxylase inhibition.

With the development of highly precise and specific computerized gas chromatography–mass spectroscopy techniques using selected ion monitoring and deuterated internal standards (GC/MS–SIM) it is now possible to assay rapidly and simultaneously the major brain monoamines DA, NA and serotonin (5-HT) together with their major metabolites (Smythe *et al.* 1982*b*). The high accuracy of this technique and its simplicity has enabled assessment of neuronal activities of the brain monoamines in relation to concurrent pituitary hormone release in broad studies of neuroendocrine control mechanisms and these studies have resulted in previously unrecognized drug effects being revealed (Smythe *et al.* 1982*a*, 1982*b*, 1982*c*, 1983).

Using the techniques of our previous studies, the present investigation was designed to examine: (i) the relative acute effects of α-MT and MIT on the concentrations and metabolism of medial basal hypothalamic DA and NA; and (ii) the relationship of these hypothalamic parameters to concurrent secretory status of pituitary prolactin (PRL) and ACTH following the administration of either α-MT or MIT to normal male rats.

Materials and Models

GC/MS–SIM Assays

The GC/MS–SIM assays for DA, NA, homovanillic acid (HVA) and 3,4-dihydroxyphenylethyleneglycol (DHPG) were carried out using a Hewlett Packard 5993A GC/MS data system (Hewlett–Packard Australia Pty Ltd, North Ryde, N.S.W.) with deuterated internal standards, and using extraction and derivatization procedures identical to those previously described (Smythe *et al.* 1983). This technique also results in the extraction and derivatization of MIT when present. Levels of MIT in the hypothalamic samples from MIT-treated animals were thus estimated from the areas under the peaks of the SIM chromatograms of the MIT specific ions (*m/z* 372 and 468) which do not occur in samples from animals not treated with MIT. However, no deuterated standard was available for the precise quantitation of MIT and the concentrations of this substance in the hypothalami were calculated by reference to a standard curve obtained from non-deuterated MIT injected separately into the GC/MS; 100% recovery was assumed. This technique is thus likely to underestimate the true levels of MIT achieved but nevertheless should provide a reasonable approximation. The between-assay coefficient of variation for the assays of DA, NA, HVA and DHPG ranged from 2·5 to 3·5%. Sensitivity was less than 200 fmol for each compound. All results are expressed as picomoles per milligram of tissue wet weight.

Reference Compounds

The results reported in this study are uncorrected and refer to the respective free bases, acids and alcohols. Each of the following reference compounds was dried *in vacuo* before use: DA hydrochloride (Calbiochem–Behring, Carlingford, Australia), L-NA (Sigma Chemical Co., St. Louis, Mo.), HVA (Sigma Chemical Co.), DHPG (Sigma Chemical Co.).

Drugs

3-Iodo-L-tyrosine and the methyl ester of α-methyl-*p*-tyrosine were obtained from Sigma Chemical Co. The doses used were the same as are commonly used to inhibit tyrosine hydroxylase activity (Scapagnini *et al.* 1970; Lemmer and Berger 1978; Smythe *et al.* 1982*a*).

Radioimmunoassays

Serum rat prolactin (rPRL) was assayed in double-antibody RIA using material supplied by Dr A. Parlow (NIAMDD, Bethesda, Maryland). Data are expressed in terms of rPRL-RP-2. The interassay coefficient of variation was less than 9%. Serum ACTH was estimated by RIA using materials supplied

by Immuno Nuclear Corporation (Stillwater, Minnesota). The limit of sensitivity for the assay was 50 pg/ml and the intra- and interassay coefficients of variation were 15% and 30% (maximum) respectively. All samples were assayed in duplicate.

Statistics

Statistical analyses were carried out using Student's *t*-test.

Animal Studies

Outbred male rats of the Wistar strain approximately 50 days old and weighing 200–220 g were used in these studies. The rats were gentled for 5 days preceding the experiments. The animals were fed *ad libitum* and were subjected to 12 h dark–12 h light cycles. The studies were conducted between 0930 and 1030 h. Eighteen animals were divided into three equal-sized groups. MIT (50 mg/kg suspended in 1 ml saline, i.p.) and α-MT (200 mg/kg, dissolved in 1 ml saline, i.p.) were administered 30 min before killing them. Saline (1 ml i.p.) was administered to animals in the control group 30 min before their death, at which time trunk blood was collected, the brains removed and the medial basal hypothalamus rapidly dissected as previously described (Smythe *et al.* 1983).

Table 1. Hypothalamic concentrations and metabolism of DA and NA and serum PRL and ACTH concentrations 30 min following the intraperitoneal administration of normal saline (controls), MIT (50 mg/kg), and α-MT (200 mg/kg)

Values are the means ± s.e.m. [a]$P<0.005$ *v.* controls; [b]$P<0.0005$ *v.* controls; [c]$P<0.01$ *v.* controls

	Controls ($n = 6$)	MIT ($n = 6$)	α-MT ($n = 6$)
DA (pmol/mg)	4·35±0·28	3·08±0·20[a]	2·62±0·19[b]
HVA (pmol/mg)	0·92±0·09	0·57±0·03[a]	0·59±0·03[a]
Ratio HVA/DA	0·21±0·01	0·19±0·01	0·22±0·01
NA (pmol/mg)	12·75±0·5	12·5±0·8	10·7±0·2[a]
DHPG (pmol/mg)	0·90±0·06	0·86±0·06	1·43±0·05[b]
Ratio DHPG/NA	0·071±0·004	0·072±0·005	0·134±0·004[b]
Serum PRL (ng/ml)	13·0±1·0	59·0±4·0[b]	35·0±7·0[c]
Serum ACTH (pg/ml)	111±11	110±19	247±13[b]

Results

Following the administration of either MIT or α-MT there were marked reductions ($P<0.005$) in the hypothalamic concentrations of both DA and HVA and highly significant increases in PRL release (see Table 1). Also shown in Table 1 are the data with respect to hypothalamic NA status and serum ACTH concentrations. α-MT, but not MIT, administration caused a highly significant fall in hypothalamic NA concentration ($P<0.005$). These changes resulted in a highly significant increase in the ratio DHPG/NA ($P<0.0005$) in the case of α-MT. Serum ACTH levels were also increased ($P<0.0005$) following α-MT but not MIT. Significant amounts of MIT were detected only in the hypothalamic samples from the MIT-treated group of rats. The mean concentration of MIT in the hypothalami of this group was estimated to be 75±25 (s.d.) pmol/mg.

Discussion

The marked reductions in hypothalamic concentrations of DA and HVA and the increased prolactin secretion following the administration of α-MT and MIT have been previously well recognized and these effects are consistent with these drugs blocking central tyrosine hydroxylase activity. The concentration of MIT achieved in the rat hypothalami 30 min following its administration in this study compares favourably with the

concentrations of this compound required to inhibit tyrosine hydroxylase activity *in vitro* (Udenfriend *et al.* 1965). The present data also show that α-MT, besides inhibiting tyrosine hydroxylase activity, exerts an unexpected acute effect of increasing hypothalamic NA metabolism which is opposite to its effect on DA metabolism. The highly significant and marked increase in the hypothalamic concentration of DHPG is evidence of an increase in hypothalamic NA neuronal activity (Warsh *et al.* 1981; Scatton 1982) as is the large increase in the ratio DHPG/NA (Smythe *et al.* 1983). That this represents a functional increase in NA activity is supported by the increase in ACTH release following α-MT administration. We have recently shown that increased hypothalamic NA neuronal activity is closely related to stress-induced ACTH (and presumably, CRF) release (Smythe *et al.* 1983) and the present data are consistent with that relationship. The stimulation of the hypothalamic–pituitary–adrenal axis by α-MT was previously interpreted in terms of the removal of an inhibitory NA effect on ACTH release (Scapagnini *et al.* 1970; Muller *et al* 1977). The possibility that some of the effects of α-MT might be stress-mediated has not been previously considered although attention has been drawn to the fact that chronic α-MT administration and the consequent central depletion of NA is not associated with ACTH–corticosterone stimulation (Muller *et al.* 1977).

The actual mechanism whereby α-MT can cause a stress response not seen with MIT remains to be determined. However, one possible explanation may relate to their markedly different major metabolic products. On the one hand MIT is metabolized to the physiological amino acid L-tyrosine but on the other α-MT gives rise to a false transmitter α-methyltyramine which could act on α-adrenoceptors to elicit increased NA firing. The possibility that the acute effects of α-MT administration on hypothalamic NA activity and ACTH release might be associated with tyrosine hydroxylase inhibition was excluded by the observation that these effects were not seen following MIT administration. The observed fall in hypothalamic NA concentration following α-MT is likely to be associated with an increase in NA utilization and not to reduced synthesis due to tyrosine hydroxylase blockade since it would not be anticipated that significant falls in NA concentration would be observed within 30 min of tyrosine hydroxylase inhibition; this is because of the significantly lower turnover rate of central NA versus DA in normal rats (G. A. Smythe, and J. E. Bradshaw, unpublished observation; cf. also Lemmer and Berger 1978). No significant change in hypothalamic NA concentration occurred within 30 min following MIT administration.

In conclusion, this study has shown that the acute administration of α-MT results in increased hypothalamic NA neuronal activity and associated effects on ACTH secretion which are unrelated to acute tyrosine hydroxylase inhibition. The data emphasize the necessity of assessing the neuronal activities of the hypothalamic monoamines following drug administration in neuroendocrine investigations so that any previously unrecognized actions of such drugs on monoamine status can be taken into account. In the absence of such data extreme caution should be exercised by investigators in interpreting the neuroendocrine actions of drugs. This investigation also indicates that, compared with α-MT, MIT is a relatively specific inhibitor of tyrosine hydroxylase and is the preferable drug in studies where acute tyrosine hydroxylase blockade is used to assess the roles of brain catecholamines.

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