Measurement of 3-methoxy-4-hydroxyphenylglycol (MHPG) in mouse brain by h.p.l.c. with electrochemical detection, as an index of noradrenaline utilisation and presynaptic α_2 -adrenoceptor function

¹D.J. Heal, M.R. Prow & W.R. Buckett

Research Department, The Boots Company PLC, Nottingham NG2 3AA

1 A novel method for measurement of 3-methoxy-4-hydroxyphenylglycol (MHPG) in mouse brain by use of high performance liquid chromatography (h.p.l.c.) with electrochemical detection is described. This technique incorporates an ethyl acetate purification procedure and uses 3-hydroxy-4-methoxyphenylglycol (iso-MHPG) as the internal standard.

2 Inhibition of monoamine oxidase by injection of tranlycypromine (5 and 10 mg kg⁻¹) or pargyline (50 and 100 mg kg⁻¹) markedly decreased brain MHPG concentrations. After injection of the tyrosine hydroxylase inhibitor, α -methyl-*p*-tyrosine (200 mg kg⁻¹), there were time-dependent linear decreases in the concentrations of noradrenaline and MHPG in mouse brain. In addition, a very good correlation ($r = 0.95$, $n = 30$; $P < 0.001$) was found between the concentrations of noradrenaline and MHPG present in the brains of the same mice after α -methyl-*p*-tyrosine treatment.

3 Mouse brain MHPG concentrations were dose-dependently reduced after administration of the α_2 -adrenoceptor agonist, clonidine (1–3000 μ g kg⁻¹), and elevated by the antagonists, idazoxan (1 and 5 mg kg⁻¹), and yohimbine (1 and 5 mg kg⁻¹). Intracerebroventricular injection of the α_1 -adrenoceptor agonist, phenylephrine (5–50 μ g) dose-dependently increased MHPG levels. The α_1 -adrenoceptor antagonist, prazosin, had no effect at the moderate dose of 1 mg kg⁻¹, but increased MHPG concentrations at 5 mg kg⁻¹. The β -adrenoceptor agonist, clenbuterol (10–1000 μ g kg⁻¹) and the antagonist, pindolol (1 and 5 mg kg⁻¹), were both without effect.

4 The decrease in brain MHPG concentrations induced by clonidine (100 μ g kg⁻¹) was prevented by prior injection of 1 mg kg⁻¹ of idazoxan or yohimbine, but not by prazosin or pindolol.

5 MHPG levels were decreased after administration of the noradrenaline reuptake inhibitor desipramine (5 and 10 mg kg⁻¹) and the non-selective monoamine reuptake inhibitors, sibutramine HCl (BTS 54 524; 1 and 3 mg kg⁻¹) and amitriptyline (5 mg kg⁻¹). However, the selective 5-hydroxytryptamine reuptake inhibitor, zimeldine (5 and 10 mg kg⁻¹), was without effect. Dexamphetamine (1 and 5 mg kg⁻¹) and methamphetamine (1 and 5 mg kg⁻¹) both decreased brain MHPG concentrations in a dose-related fashion.

6 Overall the data show that MHPG can be used as a functional index of both presynaptic α_2 -adrenoceptor activity and noradrenaline turnover and utilisation.

Introduction

In the mammalian brain, one major route by which noradrenaline can be metabolised is conversion to 3,4-dihydroxyphenylglycolaldehyde by monoamine oxidase and then to 3,4-dihydroxyphenylglycol (DHPG) by aldehyde reductase. 3-Methoxy-4-hydroxyphenylglycol (MHPG) results from methylation of DHPG by catechol-*O*-methyltransferase and

in the rat this exists as the sulphate conjugate, MHPG-SO₄ (Schanberg *et al.*, 1968; Meek & Neff, 1972). In the mouse this final step does not occur and MHPG is only found in the unconjugated form (Ceasar *et al.*, 1974). Sharman (1969) suggested that the formation of DHPG and MHPG may represent the major cerebral transformations of noradrenaline in the mouse.

Noradrenaline release in the brain is under tonic

¹ Author for correspondence.

inhibitory control by α_2 -adrenoceptors which are located on presynaptic noradrenergic nerve terminals (Heepe & Starke, 1985). However, in contrast to the situation in the peripheral nervous system, the overwhelming majority of α_2 -adrenoceptors in the brain are located on postsynaptic sites (U'Prichard *et al.*, 1980; Dooley *et al.*, 1983). Consequently, it is not possible to monitor the function of presynaptic α_2 -adrenoceptors by use of ligand-receptor binding techniques.

In view of this, a simple method for determination of MHPG in brain tissues by high performance liquid chromatography (h.p.l.c.) with electrochemical detection has been developed based on the method described for measurement of MHPG in plasma by Molyneux & Franklin (1985). The effects of administration of various α - and β -adrenoceptor agonists and antagonists on mouse brain MHPG concentrations were examined to determine whether this noradrenaline metabolite could be used as a monitor of presynaptic α_2 -adrenoceptor function. In addition, mice were also given the tyrosine hydroxylase inhibitor, α -methyl-*p*-tyrosine, to discover whether changes in MHPG concentrations reflected noradrenaline turnover.

Methods

Determination of MHPG by h.p.l.c. with electrochemical detection

Analysis of MHPG in brain samples was performed by modification of the technique described by Molyneux & Franklin (1985). Mice were killed and brains (minus cerebellum) were homogenized in 5 volumes (w/v) of ice-cold 0.1 M perchloric acid containing 0.1 μ M 3-hydroxy-4-methoxyphenylglycol hemipiperazine (iso-MHPG, internal standard) and 0.4 mM sodium metabisulphite (antioxidant) in a Polytron PT 10-35 disruptor (setting 5-6). Homogenates were centrifuged at 1100 g for 15 min at 4°C. The supernatant was then centrifuged at 11,600 g for 5 min at room temperature; 1 ml of the resulting supernatant was transferred to a 10 ml glass centrifuge tube containing 5 ml of ethyl acetate and vortex-mixed for 1 min. After centrifugation at 1300 g for 10 min at 4°C, the organic phase was transferred to a second 10 ml glass centrifuge tube containing 1 ml of 0.08 M potassium bicarbonate solution. Each tube was then vortex-mixed, centrifuged at 1300 g for 10 min at 4°C and the organic phase removed and evaporated to dryness at 30°C under a stream of nitrogen gas. The residues were dissolved in 100 μ l of h.p.l.c. mobile phase (0.1 M sodium acetate/citric acid buffer, pH 4.40 containing 8% (v/v) methanol and 4.6 mM 1-octanesulphonic acid

sodium salt) and 50 μ l was injected onto the column using a WISP 710B autoinjector (Millipore/Waters). The h.p.l.c. system consisted of a DuPont 870 pump set at 0.85 ml min⁻¹, connected to a 5 μ m Spherisorb ODS 1 reversed phase analytical column (length 250 $\times$ 4.6 mm i.d.) maintained at 45°C and protected by a Brownlee Aquapore RP300 precolumn (length 30 $\times$ 4.6 mm i.d.). MHPG and iso-MHPG were detected with a BAS LC-4B amperometric detector set at a potential of +0.75 V versus a Ag/AgCl reference electrode and linked to a DuPont SP 4100 integrator.

Measurement of noradrenaline concentrations in mouse brain by h.p.l.c. with electrochemical detection

For studies comparing the turnover rates of MHPG and noradrenaline in mouse brain after α -methyl-*p*-tyrosine administration, brains minus cerebellum were homogenized in 5 volumes (w/v) of 0.1 M perchloric acid containing 0.1 μ M iso-MHPG (internal standard MHPG assay), 0.8 μ M isoprenaline (internal standard noradrenaline assay) and 0.4 mM sodium metabisulphite (antioxidant). After centrifugation as described above, 1 ml of the supernatant was used for MHPG determination and 30 μ l was injected directly on to the h.p.l.c. system for noradrenaline determination. This comprised a DuPont 870 pump connected via a WISP 712 autoinjector to a 5 μ m Hypersil ODS 1 reversed-phase analytical column (length 250 $\times$ 4.6 mm i.d.) maintained at 45°C and protected by a Brownlee Aquapore RP 300 precolumn (length 30 $\times$ 4.6 mm i.d.). The h.p.l.c. mobile phase was 0.1 M sodium dihydrogen orthophosphate-orthophosphoric acid buffer pH 3.2 containing 16% v/v methanol, 2.8 mM 1-octanesulphonic acid sodium salt and 0.7 mM EDTA. Noradrenaline and isoprenaline were detected using a BAS LC-4B amperometric detector with a TL-5 flow-cell set at a potential of +0.75 V versus a Ag/AgCl reference electrode.

Animals and drug administration procedures

Adult male C57/B1/6 Ola mice (Olac), weighing 15-35 g were used. They were housed in groups of 5 to 10 on a 12 h light/dark cycle (commencing 07 h 00 min) at a temperature of 21°C and 55% humidity. The mice were allowed food and water *ad libitum*.

All drugs were dissolved in 0.9% (w/v) sodium chloride solution (saline), with the exception of prazosin which was dissolved in distilled water. Drugs, with the exception of α -methyl-*p*-tyrosine methyl ester and phenylephrine, were injected intraperitoneally (i.p.) in weight-related doses (0.1 ml 10 g⁻¹ body weight). For injection of phenylphrine, mice were first lightly anaesthetized with an air/halothane

mixture and phenylephrine (5–50 μg) was injected intracerebroventricularly (i.c.v.) in 2 μl of saline by use of the stereotaxic apparatus described by Heal (1984). Control mice were injected with saline (2 μl i.c.v.). α -Methyl-*p*-tyrosine (200 mg kg^{-1}) was injected intravenously via the tail veins of mice. This was because accurate estimation of noradrenaline turnover by the method of Brodie *et al.* (1966) requires immediate and complete inhibition of tyrosine hydroxylase in the brain (Costa, 1970).

Turnover parameters were calculated from the formula (see Costa, 1970).

$$\log(\text{NA}) = \log(\text{NA}_0) - 0.434 k_{\text{NA}} t$$

where

NA_0 = concentration of noradrenaline (nmol g^{-1});

NA = concentration of noradrenaline at time ' t ';

k_{NA} = fractional rate constant.

Drugs and reagents

Drugs were obtained from the following sources: clonidine HCl, 3-methoxy-4-hydroxyphenylglycol hemipiperazine (MHPG), isoprenaline HCl, pindolol, yohimbine HCl, phenylephrine HCl, tranlycypromine HCl, pargyline HCl, α -methyl-*p*-tyrosine methyl ester HCl, methamphetamine HCl (Sigma, Poole); prazosin HCl (Pfizer, Sandwich); idazoxan HCl (Reckitt and Colman, Hull); 3-hydroxy-4-methoxyphenylglycol hemipiperazine (iso-MHPG); kindly donated by Mr M. Franklin, University Department of Psychiatry, Research Unit, Littlemore Hospital, Oxford); desipramine HCl (DMI; Geigy, Horsham); zimeldine (HCl)₂ (Astra, Södertälje); amitriptyline HCl, clenbuterol HCl, sibutramine HCl (BTS 54 524; Boots, Nottingham), dexamphetamine SO_4 (Smith, Kline and French, Welwyn Garden City).

Reagents were obtained from the following sources: glacial acetic acid ('AnalaR' grade), perchloric acid ('Aristar' grade), potassium bicarbonate ('AnalaR' grade), sodium metabisulphite ('AnalaR' grade) (BDH, Poole); sodium acetate trihydrate ('HPLC' grade), citric acid ('A.R.' grade), sodium hydroxide ('A.R.' grade), 1-octane sulphonic acid sodium salt ('HPLC' grade) (Fisons, Loughborough); ethyl acetate ('HPLC' grade), methanol ('HPLC' grade) (Rathburn Chemicals, Walkerburn). All water was distilled and deionised before use.

Results

Chromatography

A chromatograph of a standard solution of MHPG and iso-MHPG (both 2.5 $\text{ng } 100 \mu\text{l}^{-1}$), obtained

Figure 1 Chromatographs of (a) a standard solution of 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3-hydroxy-4-methoxyphenylglycol (iso-MHPG) and (b) MHPG from the brain of an untreated mouse. The peaks are (1) MHPG and (2) iso-MHPG. (a) A standard solution of 2.5 ng MHPG and iso-MHPG injected in 100 μl of h.p.l.c. eluant. (b) MHPG present in the brain of an untreated mouse using 0.1 μM iso-MHPG as the internal standard for the h.p.l.c. technique. The brain MHPG concentration was 71.5 ng g^{-1} tissue wet weight. The tissue extraction procedure and chromatography technique are fully described in Methods.

using the h.p.l.c. conditions described in Methods, is shown in Figure 1a. A chromatograph of the MHPG extracted from naive mouse brain is shown for comparison (Figure 1b) and is equivalent to a tissue concentration of 71.5 ng g^{-1} wet weight. The retention times of MHPG and iso-MHPG were 7.00 and 9.30 min, respectively. The ethyl acetate extraction procedure prevented possible interference in the chromatography of MHPG and iso-MHPG by other oxidisable neurochemicals contained in brain tissue samples. However, an unidentified 'late-running' peak sometimes occurred which had a retention time of approximately 37 min. The recoveries $\pm$ s.e.mean for the ethyl acetate extractions of MHPG and iso-MHPG were not significantly different and were $27.9 \pm 0.4\%$ and $28.2 \pm 0.5\%$, respectively. Voltammograms of MHPG and iso-MHPG were almost identical and were optimum at +0.75 V versus a Ag/AgCl reference electrode. The electrochemical detector responses for MHPG and iso-MHPG were

Table 1 The effects of monoamine oxidase (MAO) inhibitors, tranylcypromine and pargyline, on 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in mouse brain

Drug	MHPG concentration (ng g ⁻¹ tissue wet wt)
Saline (0.25 ml)	73.0 ± 1.6 (6)
Tranylcypromine (5 mg kg ⁻¹)	12.7 ± 1.1 (6)***
Tranylcypromine (10 mg kg ⁻¹)	8.5 ± 0.8 (6)***
Saline (0.25 ml)	69.4 ± 2.3 (6)
Pargyline (50 mg kg ⁻¹)	17.3 ± 0.5 (6)***
Pargyline (100 mg kg ⁻¹)	20.1 ± 1.0 (6)***

Mice were given an intraperitoneal injection of tranylcypromine (5 or 10 mg kg⁻¹) or pargyline (50 or 100 mg kg⁻¹). Control mice were injected with saline (0.25 ml). Sixty minutes later, mice were killed and brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection. The results ± s.e.mean are presented, with the numbers of mice used given in parentheses. Significantly different from appropriate saline control: *** $P < 0.001$ using Student's unpaired t test.

linear over a wide concentration range (0.5–100 ng injected onto the column). With extracted brain samples the limit of detection was 2.0 ng injected onto the column (signal/noise ratio = 2 : 1).

The effects of monoamine oxidase inhibitors on mouse brain MHPG concentrations

Mouse brain MHPG concentrations were determined 60 min after administration of the monoamine oxidase (MAO) inhibitors, tranylcypromine (5 or 10 mg kg⁻¹) and pargyline (50 or 100 mg kg⁻¹). A pronounced dose-dependent decrease in the brain MHPG level was produced by tranylcypromine (Table 1). The effect of pargyline was maximal at 50 mg kg⁻¹ and increasing the dose to 100 mg kg⁻¹ did not further reduce the already markedly depleted MHPG concentrations (Table 1).

*Effects of α -methyl-*p*-tyrosine on noradrenaline turnover and MHPG concentrations in mouse brain*

After administration of α -methyl-*p*-tyrosine (200 mg kg⁻¹, i.v.), brain concentrations of noradrenaline and MHPG were measured at 0, 60, 120 and 240 min intervals after injection. There was a good correlation between the decline in the log noradrenaline concentration and time ($r = 0.85$, $n = 30$; $P < 0.001$) and this yielded a fractional rate constant (k_{NA}) of 0.11 h⁻¹ and a turnover rate of 0.28 nmol g⁻¹ wet wt h⁻¹ (Figure 2). A time-

Figure 2 The effects of α -methyl-*p*-tyrosine (α -MPT) administration on the concentrations of noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) in mouse brain. Groups of mice were killed at various time intervals after injection of α -methyl-*p*-tyrosine (200 mg kg⁻¹, i.v.) and brain concentrations of noradrenaline and MHPG were determined by separate h.p.l.c. techniques as described in Methods. Results are shown (with s.e.mean indicated by vertical bars) for observations of the decline in noradrenaline (Δ) and MHPG ($\bullet$). Linear correlation coefficients were for noradrenaline: $r = 0.85$, $n = 30$ ($P < 0.001$) and for MHPG: $r = 0.93$, $n = 30$ ($P < 0.001$).

dependent decline in log MHPG concentration was also observed ($r = 0.93$, $n = 30$; $P < 0.001$) with k_{MHPG} 0.23 h⁻¹ and a turnover rate of 0.09 nmol g⁻¹ wet wt h⁻¹ (Figure 4). There was also a very high correlation ($r = 0.95$, $n = 30$, $P < 0.001$) between the concentrations of noradrenaline and MHPG which were present in the brains of individual mice after α -methyl-*p*-tyrosine administration (Figure 3).

The effects of α - and β -adrenoceptor agonists and antagonists on MHPG concentrations in mouse brain

The effects on brain MHPG concentrations of various α - and β -adrenoceptor agonists and antagonists was determined 60 min after drug administration. The α_2 -adrenoceptor agonist clonidine (1–3000 μ g kg⁻¹) produced a dose-dependent decrease in mouse brain MHPG concentrations. The maximum reduction of 57% was observed after

Figure 3 Correlation between the concentrations of noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) in mouse brain after administration of α -methyl-*p*-tyrosine. Groups of mice were killed at various time intervals after injection of α -methyl-*p*-tyrosine (200 mg kg^{-1} , i.v.) and noradrenaline and MHPG concentrations were measured in the same animals by the two h.p.l.c. techniques described in Methods. Interval after α -methyl-*p*-tyrosine injection (min): 0 (■); 60 (▲); 120 (●); 240 (◆). Linear correlation $r = 0.94$, $n = 30$ ($P < 0.001$).

administration of $3000 \mu\text{g kg}^{-1}$ and the ED_{50} for this effect was $25 \mu\text{g kg}^{-1}$ (Figure 4). The α_1 -adrenoceptor agonist, phenylephrine ($5\text{--}50 \mu\text{g}$), dose-dependently increased brain MHPG concentrations (Table 2). However, the β -adrenoceptor agonist, clenbuterol ($10\text{--}1000 \mu\text{g kg}^{-1}$), had no effect on MHPG concentrations in mouse brain (Table 2).

Two α_2 -adrenoceptor antagonists, idazoxan (1 or 5 mg kg^{-1}) and yohimbine (1 or 5 mg kg^{-1}), both produced dose-related increases in brain MHPG concentrations (Figure 5). The α_1 -adrenoceptor antagonist, prazosin, had no effect on MHPG levels

Figure 4 Dose-response relationship for the reduction of brain 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations by clonidine administration. Brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection 60 min after injection of clonidine ($1\text{--}3000 \mu\text{g kg}^{-1}$) to mice. The inset shows the percentage decrease in response versus the clonidine dose plotted on a log scale. The ED_{50} value is $25 \mu\text{g kg}^{-1}$. Results are mean values for 5–6 mice; vertical bars show s.e.mean. The tissue extraction procedure and chromatography technique are fully described in Methods. Significantly different from saline control: ** $P < 0.01$; *** $P < 0.001$.

Table 2 The effects of phenylephrine and clenbuterol on 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in mouse brain

Drug	MHPG concentration (ng g^{-1} tissue wet wt)
Saline ($2 \mu\text{l}$ i.c.v.)	73.2 ± 1.0 (6)
Phenylephrine ($5 \mu\text{g}$ i.c.v.)	106.9 ± 4.3 (6)***
Phenylephrine ($25 \mu\text{g}$ i.c.v.)	159.2 ± 4.5 (6)***
Phenylephrine ($50 \mu\text{g}$ i.c.v.)	174.8 ± 4.3 (6)***
Saline (0.25 ml i.p.)	69.1 ± 1.3 (10)
Clenbuterol ($10 \mu\text{g kg}^{-1}$ i.p.)	69.3 ± 2.1 (6)
Clenbuterol ($100 \mu\text{g kg}^{-1}$ i.p.)	71.7 ± 1.6 (6)
Clenbuterol ($1000 \mu\text{g kg}^{-1}$ i.p.)	67.4 ± 1.7 (6)

Mice were injected intracerebroventricularly with phenylephrine ($5\text{--}50 \mu\text{g}$) or saline ($2 \mu\text{l}$). In a second series of experiments mice were given an intraperitoneal injection of clenbuterol ($10\text{--}1000 \mu\text{g kg}^{-1}$) or saline (0.25 ml). Sixty minutes later mice were killed and brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection. The results $\pm$ s.e.mean are presented, with the number of mice used given in parentheses.

Significantly different from appropriate saline control: *** $P < 0.001$ using Student's unpaired *t* test.

Figure 5 The effect of administration of various α - and β -adrenoceptor antagonists on mouse brain 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations. Mice were given an intraperitoneal injection of prazosin (1 or 5 mg kg⁻¹, b), idazoxan (1 or 5 mg kg⁻¹, c), yohimbine (1 or 5 mg kg⁻¹, d) or pindolol (1 or 5 mg kg⁻¹, e). Control mice were injected with saline (0.25 ml, a). Sixty minutes later, mice were killed and brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection. The results are presented as MHPG concentration (ng g⁻¹ tissue wet wt) with the numbers of mice used shown in each column; vertical bars show s.e.mean. For the sake of clarity saline control groups have been combined. However, data from experimental groups of mice were always compared with their own saline-treated controls which were tested at the same time. Significantly different from appropriate saline controls which were tested at the same time: **P* < 0.05; ****P* < 0.001 using Student's unpaired *t* test.

at 1 mg kg⁻¹, but did significantly elevate these at a dose of 5 mg kg⁻¹ (Figure 5). The β -adrenoceptor antagonist, pindolol (1 or 5 mg kg⁻¹), had no effect on brain MHPG concentrations at either dose (Figure 5).

The effects of α - and β -adrenoceptor antagonists on the decrease in brain MHPG concentrations induced by clonidine

After 30 min pretreatment with one of the following: prazosin (1 mg kg⁻¹), idazoxan (1 mg kg⁻¹), yohimbine (1 mg kg⁻¹), pindolol (1 mg kg⁻¹) or saline (0.25 ml), mice were injected with clonidine (100 μ g kg⁻¹), and were killed after a further 60 min and brain MHPG concentrations were determined.

Clonidine (100 μ g kg⁻¹) induced a significant fall in brain MHPG concentrations and this effect was abolished by the α_2 -adrenoceptor antagonists, idazoxan (1 mg kg⁻¹) and yohimbine (1 mg kg⁻¹) (Table 3). This reduction was not altered by pretreatment with the α_1 -adrenoceptor antagonist, prazosin (1 mg kg⁻¹) or the β -adrenoceptor antagonist, pindolol (1 mg kg⁻¹) (Table 3).

The effects of sibutramine HCl and various other monoamine reuptake inhibitors on brain MHPG concentrations

Mice were injected with sibutramine HCl (1 or 3 mg kg⁻¹), DMI (5 or 10 mg kg⁻¹), amitriptyline (5 or 10 mg kg⁻¹), zimeldine (5 or 10 mg kg⁻¹) or saline (0.25 ml). Sixty minutes later, mice were killed and brain MHPG concentrations were determined.

Table 3 The effects of α - and β -adrenoceptor antagonists on the clonidine-induced decrease in mouse brain 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations

First injection	Second injection	Brain MHPG concentration (ng g ⁻¹ tissue wet wt) ± s.e.
Saline (0.25 ml)	Saline (0.25 ml)	73.5 ± 1.0 (17)
Saline (0.25 ml)	Clonidine (100 μ g kg ⁻¹)	49.7 ± 0.9 (18)***
Prazosin (1 mg kg ⁻¹)	Clonidine (100 μ g kg ⁻¹)	48.8 ± 1.4 (6)***
Idazoxan (1 mg kg ⁻¹)	Clonidine (100 μ g kg ⁻¹)	65.4 ± 3.1 (6)†††
Yohimbine (1 mg kg ⁻¹)	Clonidine (100 μ g kg ⁻¹)	72.1 ± 0.6 (6)†††
Pindolol (1 mg kg ⁻¹)	Clonidine (100 μ g kg ⁻¹)	43.7 ± 1.7 (6)***

Mice were given an intraperitoneal injection of prazosin (1 mg kg⁻¹), idazoxan (1 mg kg⁻¹), yohimbine (1 mg kg⁻¹) or pindolol (1 mg kg⁻¹). Control mice received saline (0.25 ml). Thirty minutes later, mice were injected with clonidine (100 μ g kg⁻¹, i.p.) or saline (0.25 ml, i.p.) and killed after a further 60 min. Brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection. The results ± s.e.mean are presented, with the numbers of mice used given in parentheses. For the sake of clarity saline/saline and saline/clonidine control groups have been combined. However, data from experimental groups of mice were always compared with their own control groups of mice treated with saline/saline or saline/clonidine which were tested at the same time.

Significantly different from appropriate saline/saline control: ****P* < 0.001 using Student's unpaired *t* test.

Significantly different from appropriate saline/clonidine control: †††*P* < 0.001 using Student's unpaired *t* test.

Table 4 The effects of sibutramine HCl and other monoamine reuptake inhibitors on 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in mouse brain

Drug	MHPG concentration (ng g ⁻¹ tissue wet wt)	
	Saline control	Drug-treated
Sibutramine HCl (1 mg kg ⁻¹)	79.2 ± 1.9 (6)	63.0 ± 3.8 (6)**
Sibutramine HCl (3 mg kg ⁻¹)	79.2 ± 1.9 (6)	52.9 ± 3.5 (6)***
DMI (5 mg kg ⁻¹)	74.2 ± 2.4 (6)	58.1 ± 2.1 (5)**
DMI (10 mg kg ⁻¹)	74.2 ± 2.4 (6)	52.9 ± 1.9 (6)***
Amitriptyline (5 mg kg ⁻¹)	78.6 ± 1.7 (6)	69.0 ± 1.7 (6)**
Amitriptyline (10 mg kg ⁻¹)	78.6 ± 1.7 (6)	74.8 ± 7.1 (6)
Zimeldine (5 mg kg ⁻¹)	61.5 ± 3.9 (6)	58.2 ± 2.7 (6)
Zimeldine (10 mg kg ⁻¹)	61.5 ± 3.9 (6)	58.8 ± 1.9 (6)

Mice were given an intraperitoneal injection of the monoamine reuptake inhibitors: sibutramine HCl (1 or 3 mg kg⁻¹), desipramine (DMI; 5 or 10 mg kg⁻¹), amitriptyline (5 or 10 mg kg⁻¹) or zimeldine (5 or 10 mg kg⁻¹). Control mice were injected with saline (0.25 ml). Sixty minutes later, mice were killed and brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection. The results ± s.e.mean are presented, with the numbers of mice used given in parentheses.

Significantly different from appropriate saline control group ** $P < 0.01$; *** $P < 0.001$ using Student's unpaired t test.

The non-selective monoamine reuptake inhibitor, sibutramine HCl, induced a dose-related decrease in brain MHPG concentrations (Table 4). The selective noradrenaline reuptake inhibitor, DMI, also caused a dose-related decrease in MHPG levels. Amitriptyline, another non-selective monoamine reuptake inhibitor, decreased brain MHPG concentrations at 5 mg kg⁻¹, but had no effect at 10 mg kg⁻¹. The selective 5-HT reuptake inhibitor, zimeldine (5 or 10 mg kg⁻¹), had no effect on brain MHPG concentrations (Table 4).

The effects of dexamphetamine and methamphetamine on brain MHPG concentrations

Mice were injected with dexamphetamine (1 or 5 mg kg⁻¹), methamphetamine (1 or 5 mg kg⁻¹) or saline (0.25 ml). Sixty minutes later, mice were killed and brain MHPG concentrations were determined. Dexamphetamine and methamphetamine both induced dose-related decreases in MHPG concentrations in mouse brain (Table 5).

Discussion

A sensitive and selective method for determination of free MHPG concentrations in mouse brain by use of h.p.l.c. with electrochemical detection is described. This procedure incorporates a simple ethyl acetate extraction procedure. Iso-MHPG was used as the internal standard for this technique and extraction recoveries of MHPG and iso-MHPG were identical. In addition, the electrochemical profiles of MHPG and iso-MHPG were also very similar with

maximum oxidation currents being observed at +0.75 V versus a Ag/AgCl reference electrode. There was no chromatographic interference from other oxidisable biological material in brain extracts, although an unidentified late-running peak did sometimes occur with a retention time of approximately 37 min. This procedure was based on the method previously described by Molyneux & Franklin (1985) for measurement of MHPG concentrations

Table 5 The effects of dexamphetamine and methamphetamine on 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in mouse brain

Drug	MHPG concentration (ng g ⁻¹ tissue wet wt)
Saline (0.25 ml)	73.0 ± 3.4 (6)
Dexamphetamine (1 mg kg ⁻¹)	63.6 ± 1.7 (6)*
Dexamphetamine (5 mg kg ⁻¹)	57.2 ± 2.2 (6)**
Saline (0.25 ml)	76.9 ± 1.9 (6)
Methamphetamine (1 mg kg ⁻¹)	67.2 ± 2.4 (6)*
Methamphetamine (5 mg kg ⁻¹)	66.2 ± 3.4 (6)*

Mice were given an intraperitoneal injection of dexamphetamine (1 or 5 mg kg⁻¹) or methamphetamine (1 or 5 mg kg⁻¹). Control mice were injected with saline (0.25 ml). Sixty minutes later, mice were killed and brain MHPG concentrations were determined by h.p.l.c. with electrochemical detection. The results ± s.e.mean are presented, with the numbers of mice used given in parentheses.

Significantly different from appropriate saline control group: * $P < 0.05$; ** $P < 0.01$ using Student's unpaired t test.

in human plasma samples. Apart from the obvious differences between the tissue matrices involved, the major distinction was the use of an amperometric, rather than a coulometric detector, in the procedure described in this paper. The coulometric detection system has the ability to eliminate background noise from the solvent system and remove potentially interfering substances by complete oxidation or reduction at the first of its two detection electrodes. However, in our hands, no problems were encountered when using the less sophisticated and selective, but more ubiquitous, amperometric detection system.

In an earlier study in this laboratory, an h.p.l.c.-electrochemical detection assay for MHPG determination in mouse brain was developed which used phenyl bonded silica-gel mini-columns for sample purification (Buckett & Diggory, 1985). However, no internal standard was incorporated in this method to correct for differences in recovery. Moreover, iso-MHPG cannot be used as the internal standard for this procedure, because of the marked difference in the recoveries of this internal standard and MHPG (Prow & Heal, unpublished observations).

In brain, noradrenaline is initially metabolized to 3,4-dihydroxyphenylglycolaldehyde by MAO before subsequent conversion to MHPG by aldehyde reductase and catechol-*O*-methyltransferase. When this metabolic route is blocked by administration of the MAO inhibitors, tranlylcypromine and pargyline, this produced a marked and rapid decrease in the brain's concentrations of MHPG confirming the earlier reports by Ceasar *et al.* (1974) and Buckett & Diggory (1985).

After inhibition of tyrosine hydroxylase, with α -methyl-*p*-tyrosine, there was a good linear correlation between the reduction of log noradrenaline concentration and time. A similar linear relationship with time was also found for MHPG concentrations in the brain. Although the fractional rate constant for MHPG was approximately twice that for noradrenaline, the turnover rate was only 15% of that of the parent monoamine, reflecting the low brain concentrations of MHPG with respect to noradrenaline. However, a very good correlation was found between the concentration of noradrenaline and MHPG in the brains of individual mice after administration of α -methyl-*p*-tyrosine. These results suggest that MHPG levels in mouse brain may provide a useful index of noradrenaline turnover.

It is well established that noradrenaline release in the brain is under tonic inhibitory control by presynaptic α_2 -adrenoceptors located on noradrenaline terminals (Schoffelmeeer & Mulder, 1983; Heepe & Starke, 1985). It is suggested that functional release and utilisation of brain noradrenaline is reflected by the formation of MHPG in mice (Ceasar *et al.*, 1974)

and MHPG-SO₄ in rats (Meek & Neff, 1972). In this investigation, the α_2 -adrenoceptor agonist, clonidine, was found to decrease dose-dependently brain MHPG concentrations in mice and the ED₅₀ for this effect was 25 $\mu\text{g kg}^{-1}$. This agrees with the hypothesis of Andén *et al.* (1976) that, at doses up to 100 $\mu\text{g kg}^{-1}$, clonidine preferentially stimulates presynaptic α_2 -adrenoceptors in the brain. Conversely, administration of the α_2 -antagonists, idazoxan and yohimbine, markedly elevated MHPG levels and identical effects have previously been reported for mianserin (Buckett & Diggory, 1985), another drug which is an α_2 -adrenoceptor antagonist (Baumann & Maitre, 1977; Pommier *et al.*, 1982). Hence, α_2 -adrenoceptor agonists and antagonists produce opposite effects on MHPG formation, in agreement with the hypothesis that noradrenaline release is tonically controlled by presynaptic α_2 -adrenoceptors (Schoffelmeeer & Mulder, 1983; Heepe & Starke, 1985). The actions of the drugs acting at α_1 -adrenoceptors on brain MHPG levels were more complex. Intracerebroventricular administration of the agonist, phenylephrine, produced a marked dose-dependent increase in brain MHPG concentrations. Previously, Heal (1984) demonstrated that central administration of phenylephrine to C57/B1/6 mice caused marked behavioural excitation and increased locomotor activity at these doses and, furthermore, that this response was mediated by at least one other noradrenergic system 'beyond' the α_1 -adrenoceptors. Hence, the increase in MHPG concentration which follows phenylephrine administration provides further evidence to confirm that other noradrenaline neurones are involved in the generation of this behavioural syndrome. The α_1 -adrenoceptor antagonist, prazosin, had no effect on MHPG concentrations at 1 mg kg^{-1} , but increased them by 21% at 5 mg kg^{-1} . This effect was not likely to result from non-selective inhibition of α_2 -adrenoceptors, because prazosin has greater than 250 fold α_1/α_2 selectivity in the brains of C57/B1/6 mice (Heal *et al.*, 1987). However, the prazosin dose of 5 mg kg^{-1} is greater than that required to abolish α_1 -adrenoceptor-mediated activity in these mice (Heal, 1984) and the elevated MHPG levels may result from increased noradrenergic firing which is modulated via a long neuronal feed-back loop.

Sibutramine HC1 and amitriptyline, which inhibit noradrenaline and 5-hydroxytryptamine (5-HT) reuptake, and DMI, which is selective for noradrenaline, all reduced levels of MHPG in the brain. This reduction was probably the result of two distinct mechanisms. Firstly, the increased synaptic levels of noradrenaline presynaptic activated α_2 -adrenoceptors which decreased noradrenaline release. Secondly, the potent reuptake inhibition prevented noradrenaline from re-entering the presynaptic terminal

for subsequent metabolism by monoamine oxidase (MAO). Amitriptyline and sibutramine HCl were unlikely to have altered brain MHPG concentrations by their effects on 5-HT reuptake, because the selective 5-HT reuptake inhibitor, zimeldine (Ross *et al.*, 1976), had no effect on brain levels of this noradrenaline metabolite. The failure of amitriptyline to decrease MHPG concentrations at the higher dose tested was probably due to inhibition of terminal autoreceptors, because this antidepressant is also a moderately potent α_2 -adrenoceptor antagonist (Maggi *et al.*, 1980). By contrast, α_2 -adrenoceptor antagonist activity is not possessed by either sibutramine HCl (Heal and Buckett, unpublished observation) or DMI (Maggi *et al.*, 1980). Dexamphetamine and methamphetamine also produced dose-related decreases in MHPG formation. While this may appear to be at variance with their actions on noradrenaline release and function, it has been suggested that the pharmacological effects of amphetamine on noradrenaline are more predominantly the result of reuptake inhibition than release (Von Voigtlander & Moore, 1973; Raiteri *et al.*, 1975).

In rat brain, MHPG mainly exists as the sulphate conjugate (Schanberg *et al.*, 1968; Meek & Neff, 1972) and it has been suggested that this noradrenaline metabolite is a good index of the functional uti-

lisation of noradrenaline (Meek & Neff, 1972). In addition, the inhibitory actions of clonidine on MHPG formation have been widely used as an index of presynaptic α_2 -adrenoceptor function (McMillen *et al.*, 1980; Sugrue, 1981; 1982). The results presented in this study support the hypothesis of Ceasar *et al.* (1974) and Buckett & Diggory (1985) that MHPG concentrations in mouse brain reflect noradrenaline utilisation after a wide variety of pharmacological manipulations. Furthermore, the data also indicate that the clonidine-induced decrease in MHPG can be used as a model of presynaptic α_2 -adrenoceptor function in mouse brain. Since MHPG, but not MHPG-SO₄, can be simply and sensitively determined by h.p.l.c. with electrochemical detection, this highlights the viability of using mice rather than rats for these types of study.

In conclusion, a simple, sensitive and selective method for measuring free MHPG in mouse brain is described. This noradrenaline metabolite can be used as an index of either noradrenaline utilisation or presynaptic α_2 -adrenoceptor function.

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