temperature responses to (+)-amphetamine injection on the 1st, 12th and 20th days (Fig. 1). Brain noradrenaline and dopamine concentrations were 27 and 19% of control values in the 6-OH-DA-treated rats, the control values being 627 ± 53 and 960 ± 36 ng g^{-1} respectively.

According to these results it would appear that (+)-amphetamine-induced hyperthermia may be due to some central catecholaminergic mechanisms rather than peripheral mechanisms, because, as is clearly shown in Fig. 1, the selective elimination of central catecholamines by intraventricular 6-OH-DA facilitated the tolerance development to the hyperthermic effect of (+)-amphetamine. However, we do not know which of these amines, noradrenaline or dopamine, plays a predominant role in the development of hyperthermia. Some workers have attributed the amphetamine-induced hyperthermia to stimulation of dopaminergic receptors in the central nervous system in rats (Matsumato & Griffin, 1971) and rabbits (Hill & Horita, 1971).

We would like to acknowledge the technical assistance of Mr. V. Baloğlu. I. H. Ulus thanks the Yurtoglu Ltd Drug company for financial support.

Department of Pharmacology and Clinical Pharmacology,
Bursa Medical School, Bursa, Turkey.

I. H. ULUS
B. K. KIRAN

October 8, 1974

REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–375.

BRESSE, G. R. & TRAYLOR, T. D. (1970). *Ibid.*, **174**, 413–420.

BRODIE, B. B., CHO, A. K., STEFANO, F. J. E. & GESSA, G. L. (1969). In: *Advances in Biochemcial Psychopharmacology*, Vol. 1 pp. 219–238. Editors: Costa, E. and Greengard, P. New York.

FIBIGER, H. C., FIBIGER, H. P. & ZIS, A. P. (1973). *Br. J. Pharmac.*, **47**, 683–692.

HILL, H. F. & HORITA, A. (1971). *J. Pharm. Pharmac.*, **23**, 715–717.

MATSUMATO, C. & GRIFFIN, W. (1971). *Ibid.*, **23**, 710.

MATSUMATO, C. & SHAW, W. N. (1971). *Ibid.*, **23**, 387–388.

NOBLE, E. P., WURTMAN, R. J. & AXELROD, J. (1967). *Life Sci.*, **6**, 281–191.

URETSKY, N. J. & IVERSEN, L. L. (1970). *J. Neurochem.*, **17**, 269–278.

WEIL-MALHERBE H. (1972). In: *Methods of Biochemical Analysis, Suppl: Analysis of Biogenic Amines and Their Related Enzymes*, p. 133. Editor, Glick, D. New York. Wiley-Interscience.

The influence of desipramine and amitriptyline on the accumulation of [^{3}H]noradrenaline and its two major metabolites formed from [^{3}H]tyrosine in the rat brain

The tricyclic antidepressant drug desipramine, in contrast to amitriptyline, is a potent inhibitor of the neuronal uptake of noradrenaline into central noradrenergic neurons (Glowinski, Axelrod & Iversen, 1966; Giese, Rüther & Matussck, 1967; Carlsson, Corrodi & others, 1969; Schildkraut, Schanberg & others, 1967; Schildkraut, Draskoczy & others, 1971; Squires, 1974). It has also been shown, that desipramine produces a decreased accumulation of labelled noradrenaline synthesized from tyrosine without affecting the endogenous noradrenaline level (Nybäck, Borzecki & Sedvall, 1968; Schubert, Nybäck & Sedvall, 1970; Nielsen, Eplov & Scheel-Krüger, 1974). Amitriptyline produces only a weak or no effect on noradrenaline accumulation from labelled tyrosine (Schubert & others, 1970). The present investigation was made to clarify the influence of desipramine and amitriptyline on the metabolism of brain [^{3}H]noradrenaline (^{3}H-NA) synthesized from intravenously or intraventricularly injected [^{3}H]tyrosine.

Previously we have found that conjugates of the two neutral metabolites, 3-methoxy-4-hydroxyphenylglycol (MOPEG) and 3,4-dihydroxyphenylglycol (DOPEG), were both quantitatively the major metabolites of noradrenaline in the rat brain (Nielsen & others, 1974; Braestrup, Nielsen & Scheel-Krüger, 1974).

It has also been shown that alterations in noradrenergic nerve activity in the cns can be reflected in the level of endogenous MOPEG (Korf, Aghajanian & Roth, 1973; Walter & Eccleston, 1973).

The present results were obtained according to a newly developed biochemical procedure which permits the measurement of labelled noradrenaline and its major metabolites ^{3}H-MOPEG (free + conjugated), ^{3}H-DOPEG (conjugated) in the rat brain after intravenous or intraventricular injection of [^{3}H]tyrosine (Nielsen to be published).

Male Wistar rats about 270 g were injected with either saline or a tricyclic antidepressant drug subcutaneously 30 min before injection of [^{3}H]tyrosine (50–53 Ci mmol^{-1}) either intraventricularly (50 μCi in 15 μl Merles solution) or intravenously (325 μCi in 150 μl saline). The rats were decapitated 2 h after the injections of [^{3}H]tyrosine. The brains were homogenized in N acetic acid (Braestrup & others, 1974; Nielsen & others, 1974). ^{3}H-NA and [^{3}H]dopamine were absorbed on alumina, eluted in 0·5 N acetic acid and separated on an Amberlite CG 120 column. The supernatant from the alumina was passed through an Amberlite CG 120 column at pH 2 for absorption of [^{3}H]tyrosine. The effluent and washings from this column containing conjugated DOPEG and free + conjugated MOPEG were incubated with glusulase for hydrolysing. After overnight incubation, ^{3}H-MOPEG and ^{3}H-DOPEG were extracted into ethyl acetate (pH 7) and separated by thin-layer chromatography (t.l.c.) in the solvent system, chloroform–glacial acetic acid–water (2:2:1 by vol). ^{3}H-MOPEG was further separated from unknown labelled components by extraction from the cellulose MN 300 powder at R_F 0·60 into methanol, and the methanol extract was then chromatographed in a second solvent system, n-butanol–methanol–N formic acid (3:1:1 by vol).

The biochemical analyses (Table 1) showed that the pretreatment with desipramine (2·5 or 20 mg kg^{-1}) leads to a decreased formation of ^{3}H-NA and its two labelled metabolites MOPEG and DOPEG after the intravenous injection of [^{3}H]tyrosine. This strong indication of inhibition of total brain noradrenaline synthesis was not found after a high dose of amitriptyline, 20 mg kg^{-1}.

Table 1. *Effect of desipramine and amitriptyline on ^{3}H-NA synthesis and metabolism [^{3}H]tyrosine in rat brain. Saline or drugs were administered subcutaneously 30 min before intravenously injected ^{3}H-tyrosine (4·4 μg kg^{-1}).* Animals were killed 2 h after [^{3}H]tyrosine. Figure for saline treated animals represents d. min^{-1} g^{-1} brain tissue ± s.e.m. (n), while values of drug-treated animals are denoted in percentage of paired control values (n).

Treatment	Dose mg kg^{-1}	^{3}H-NA	^{3}H-MOPEG §	^{3}H-DOPEG §	^{3}H-DA	[^{3}H]Tyrosine
Desipramine	2·5	74 ± 5 (4) ***	75 ± 7 (4) **	73 ± 8 (4) **	126 ± 8 (4) *	91 ± 7 (3)
Desipramine	20	67 ± 6 (4) ***	66 ± 6 (4) ***	63 ± 11 (4) **	131 ± 22 (4)	131 ± 9 (4) ***
Amitriptyline	20	89 ± 6 (4)	96 ± 15 (4)	121 ± 13 (4)	119 ± 24 (4)	111 ± 12 (4)
Saline (values are absolute in d. min^{-1} g^{-1})		3565 ± 123 (12)	915 ± 52 (11)	625 ± 81 (12)	6930 ± 348 (8)	57100 ± 38 (7)

*P<0·05; **P<0·02; ***P<0·01

§^{3}H-MOPEG is the sum of free plus conjugated ^{3}H-MOPEG; ^{3}H-DOPEG is conjugated ^{3}H-DOPEG.

Table 2. *Effect of desipramine and amitriptyline on ^{3}H-NA synthesis and metabolism from [^{3}H]tyrosine in rat brain.* Saline or drugs were administered subcutaneously 30 min before intraventricular injection of [^{3}H]tyrosine (50μCi). Animals were killed 2 h after [^{3}H]tyrosine. Figures for saline treated animals represent d. min^{-1} g^{-1} brain tissue ± s.e.m. (n) while values of drug treated animals are denoted in percentage of paired control values (n).

Treatment	Dose mg kg^{-1}	^{3}H-NA	^{3}H-MOPEG §	^{3}H-DOPEG §	^{3}H-DA	[^{3}H]Tyrosine
Desipramine	2·5	90 ± 8 (7)	72 ± 7 (7) **	61 ± 7 (7) **	112 ± 9 (7)	117 ± 15 (7)
Desipramine	20	74 ± 6 (6) **	64 ± 6 (6) **	48 ± 6 (6) ***	110 ± 11 (6)	121 ± 10 (6)
Amitriptyline	10	86 ± 6 (4) *	104 ± 7 (4)	108 ± 7 (4)	102 ± 27 (4)	82 ± 7 (4)
Saline		39400 ± 1650 (13)	6165 ± 330 (13)	7066 ± 421 (13)	19400 ± 1700 (12)	373000 ± 21600 (13)

*$P<0·05$; **$P<0·01$; ***$P<0·01$.
§^{3}H-MOPEG is the sum of free plus conjugated ^{3}H-MOPEG; ^{3}H-DOPEG is conjugated 3H-DOPEG.

A similar conclusion was obtained after the intraventricular injection of [^{3}H]-tyrosine, since desipramine (2·5 or 20 mg kg^{-1}), but not amitriptyline (10 mg kg^{-1}) decreased the brain concentrations of ^{3}H-MOPEG and ^{3}H-DOPEG, (Table 2). However, the brain concentrations of ^{3}H-NA showed less pronounced decreases after the intraventricular injections compared with the intravenous administration. Desipramine, 2·5 mg kg^{-1}, therefore induced no significant decrease in ^{3}H-NA, whereas at 20 mg kg^{-1} it produced a significant ($P < 0·01$) decrease.

The present results provide strong direct evidence that the secondary amine desipramine but not the tertiary amine amitriptyline decreases total brain synthesis of noradrenaline, since both ^{3}H-NA and its major metabolites ^{3}H-MOPEG and ^{3}H-DOPEG were decreased after desipramine.

This effect may be related to the induction of a negative feed-back mechanism induced by an increased activity of noradrenaline after uptake inhibition by desipramine (see also Nybäck & others, 1968; Schubert & others, 1970). In support of this conclusion, we have found that clonidine, which directly activates central noradrenaline receptors, produces effects similar to those of desipramine on accumulation of labelled dopamine, noradrenaline, MOPEG and DOPEG after intraventricularly injected [^{3}H]tyrosine in the rat brain.

In treatments when desipramine was given before intraventricularly injected [^{3}H]-tyrosine, there was a tendency for ^{3}H-DOPEG, the catechol-deaminated noradrenaline metabolite to be more decreased than ^{3}H-NA and ^{3}H-MOPEG (Table 2) indicating that this hitherto neglected major metabolite of noradrenaline may provide valuable information on the mechanism by which drugs affect central noradrenergic transmission.

This work was supported by grants from Foundations' Fund for Research in Psychiatry, New Haven, U.S.A., and by a grant from Carlsberg Fund, Copenhagen. The skilful technical assistance of Mrs. J. Tarnøe and Mrs. Aa. Krath is highly appreciated.

Sct. Hans Hospital,
Central Laboratory, Dept. E.,
DK-4000 Roskilde, Denmark.

M. NIELSEN

October 22, 1974

REFERENCES

BRAESTRUP, C., NIELSEN, M. & SCHEEL-KRÜGER, J. (1974). *J. Neurochem.*, **23**, 569–578.

CARLSSON, A., CORRODI, H., FUXE, K. & HÖKFELT, T. (1969). *Eur. J. Pharmac.*, **5**, 367–374.

GIESE, J., RÜTHER, E. & MATUSSEK, N. (1967). *Life Sci.*, **6**, 1975–1932.

GLOWINSKI, J., AXELROD, I. & IVERSEN, L. L. (1966). *J. Pharmac. exp. Ther.*, **153**, 30–41.

KORF, J., AGHAJANIAN, G. K. & ROTH, R. H. (1973). *Eur. J. Pharmac.*, **21**, 305–310.

NIELSEN, M., EPLOV, L. & SCHEEL-KRÜGER, J. (1974). *Arch. Pharmac.*, **285**, 15–18.

NYBÄCK, H., BORZECKI, Z. & SEDVALL, G. (1968). *Eur. J. Pharmac.*, **4**, 395–403.

SCHILDKRAUT, J. J., SCHANBERG, S. M., BREESE, G. R. & KOPIN, I. J. (1967). *Am. J. Psychiat.*, **124**, 600–608.

SCHILDKRAUT, J. J., DRASKOCZY, P. R., GERSHON, E. S., REICH, P. & GRAB, E. L. (1971). *Brain Chemistry and Mental Disease*, Editors: Ho, B. T. and McIsaac, W. M. pp. 215–236. New York: Plenum Publ. Corp.

SCHUBERT, J., NYBÄCK, H. & SEDVALL, G. (1970). *J. Pharm. Pharmac.*, **22**, 136–139.

SQUIRES, R. (1974). *Ibid.*, **26**, 364–366.

WALTER, D. S. & ECCLESTON, D. (1973). *J. Neurochem.*, **21**, 281–289.

The composition of cetostearyl alcohol

Cetostearyl alcohol is a material widely used in the pharmaceutical industry as a component of creams, ointments and emulsifying waxes. Variations in the behaviour of cetostearyl alcohol in production processes led to determination of its hydrophilic-lipophilic character by the method of Greenwald, Brown & Fineman (1956). Different batches, when titrated with water, in a benzene dioxane system gave different cloud points, presumably as a result of variable composition.

Examination of the cetyl and stearyl alcohol contents by gas-liquid chromatography on a 5 ft, 5% OV17 column at 190°, showed no correlation with the cloud point. However in many batches the total cetyl plus stearyl alcohol content amounted to only 80–85% w/w of the total. Even if myristyl alcohol, another normal component, was included, the total was often still below 90%.

The British Pharmacopoeia monograph for cetostearyl alcohol contains a number of limit tests which control the quantities of other classes of compounds which could be present. Fatty acids are controlled by titration with 0·1 N sodium hydroxide to a limit of 0·14% w/w calculated as stearic acid. Esters are controlled by the Saponification Value to a limit of less than 0·01% w/w in terms of methyl stearate. Unsaturated components are controlled by the Iodine Value to a limit of 3% w/w in terms of oleoyl alcohol. However in our experience of this determination this value rarely exceeds the equivalent of 1% w/w. Hydrocarbons are controlled gravimetrically after a chromatographic separation on alumina; the limit corresponds to 1·5% w/w. Hydrocarbons are frequently present in commercial material at levels exceeding 1% w/w, however none of these components can account for the missing 10% of the total.

Examination of the gas chromatographic traces used for the analysis of the cetyl and stearyl alcohols revealed a number of extra peaks. To improve the resolution of these compounds a 13 ft 9% OV 101 column was specially constructed and used with temperature programming from 160° at 6° min^{-1} to 300° (Fig. 1). At higher sensitivity more components are detected. Peaks equivalent to more than 0·01% w/w of the mixture account for approximately 99% of the total. Thirty-six batches from five different suppliers were examined by this means. Components were identified initially by comparison of the retention times of the parent compounds and