Br. J. Pharmac. (1979), **65**, 489–494

THE EFFECT OF PARGYLINE AND DESMETHYLIMIPRAMINE ON MONOAMINE CONCENTRATIONS AND AMPHETAMINE-INDUCED GLYCOGENOLYSIS IN THE MOUSE BRAIN

D.A. HUTCHINS

Department of Pharmacology, School of Pharmacy, Leicester Polytechnic, Leicester LE1 9BH

1 Pargyline (100 mg/kg) increased the concentration of cerebral noradrenaline dopamine and 5-hydroxytryptamine in the mouse. Amphetamine (5 mg/kg) reduced the concentration of noradrenaline and increased the concentrations of 5-hydroxytryptamine and dopamine.

2 When amphetamine was administered 4 h after an injection of pargyline, the effect of the sympathomimetic drug on the concentrations of noradrenaline and 5-hydroxytryptamine was not altered. The effect on the dopamine content was reversed, amphetamine causing a decrease instead of an increase.

3 Pargyline increased the concentration of cerebral glycogen, whereas amphetamine caused a decrease.

4 The administration of amphetamine 4 h after pargyline resulted in a decrease in brain glycogen similar to that seen after amphetamine alone.

5 These results suggest that the potentiation of the effect of amphetamine on animal behaviour by pretreatment with an inhibitor of monoamine oxidase is not mediated through a central action on noradrenaline release.

6 Amphetamine-induced glycogenolysis was antagonized by 71% by desmethylimipramine (10 mg/kg).

7 The change in glycogen concentration as a function of time after an injection of amphetamine was not modified when 2 consecutive doses of amphetamine were given with an interval between doses of 30 minutes.

Introduction

The administration of amphetamine to the mouse results in an initial decrease in the concentration of brain glycogen (Hutchins & Rogers, 1970; Rogers & Hutchins, 1972) which can be antagonized by the prior injection of a β-adrenoceptor blocking drug (Hutchins & Rogers, 1971) or drugs that deplete the catecholamines of the central nervous system (CNS) (Hutchins & Rogers, 1973). It is possible therefore that amphetamine-induced glycogenolysis in the mouse brain is mediated by the release of catecholamines from central nerve endings, the extracellular catecholamines then being available to stimulate the β-adrenoceptors of the brain and activate the adenyl cyclase system. Depletion of central catecholamines by inhibition of their synthesis or storage is likely to result in a reduced amount of the monoamine available for release and this leads to a diminution in the effect of amphetamine on the concentration of brain glycogen (Hutchins & Rogers, 1973). Conversely, inhibition of the metabolism or re-uptake of the catecholamines is likely to increase the amount of monoamine potentially available for release, or stimulation of receptors and this might lead to a potentiation of the cerebral glycogenolytic effect of amphetamine.

In this paper the effect of amphetamine on the concentration of the monoamines, noradrenaline, dopamine and 5-hydroxytryptamine and on the concentration of glycogen in the brains of mice treated with pargyline has been determined. The effect of desmethylimipramine (DMI) and a consecutive dose of amphetamine on amphetamine-induced glycogenolysis has also been examined.

Methods

Experiments were performed on male albino mice weighing 20 to 30 g. The mice were kept in a laboratory with a 12 h light and 12 h dark cycle (06 h

0007-1188/79/030489-06 $01.00

00 min to 18 h 00 min light) and were allowed free access to food and water before and during experiments. The environmental temperature was maintained at 20 to 22°C.

In order to minimize the effect of the circadian rhythm in the concentration of brain glyocen (Hutchins & Rogers, 1970) the injections were given at a time such that the experimental mice were killed within 1 h of the control animals during the late evening.

Estimation of brain glycogen

The mice were killed by immersion in liquid nitrogen and the brains were chiselled out of the skull whilst in the deeply frozen state. Each brain was weighed rapidly before crushing on a stainless steel anvil cooled with liquid nitrogen. One mouse brain was used for each estimation. Cerebral glycogen was determined by a modification (Hutchins & Rogers, 1970) of the method of Le Baron (1955).

Estimation of cerebral monoamines

Mice were killed by decapitation and the brains from two animals were pooled and frozen in liquid nitrogen until used for assay. The monoamines were extracted simultaneously by the method of Shore & Olin (1958). Aliquots of the final acid extract were used for the assay of noradrenaline (Anton & Sayre, 1962), dopamine (Carlsson & Waldeck, 1958) and 5-hydroxytryptamine (Bogdanski, Pletscher, Brodie & Udenfriend, 1956).

Body temperature

Body temperature was measured by means of an electric thermometer (Light Laboratories, Brighton) and an oesophageal probe (Brittain & Spencer, 1964).

Drugs

The drugs used were (+)-amphetamine sulphate, pargyline hydrochloride and desmethylimipramine (DMI). They were dissolved in distilled water and injected in a volume of 1.0 ml/100 g body weight. Doses are expressed in terms of the salt where these were used.

Results

Pargyline-amphetamine

Mice in groups of 4 to 5 were injected intraperitoneally with pargyline hydrochloride (100 mg/kg); 4 h after this injection amphetamine sulphate (5 mg/kg) was administered by the same route and the mice were killed at suitable time intervals thereafter. Animals in the control groups were injected with pargyline and killed at various times up to 8 h after injection, or treated with amphetamine and killed 30 or 60 min after injection.

The concentrations of noradrenaline, dopamine and 5-hydroxytryptamine in the brain were increased for at least 8 h after treatment with pargyline (Figure 1). An injection of amphetamine resulted in a decrease in the concentration of noradrenaline and increase in the concentrations of dopamine and 5-hydroxytryptamine. The administration of pargyline 4 h

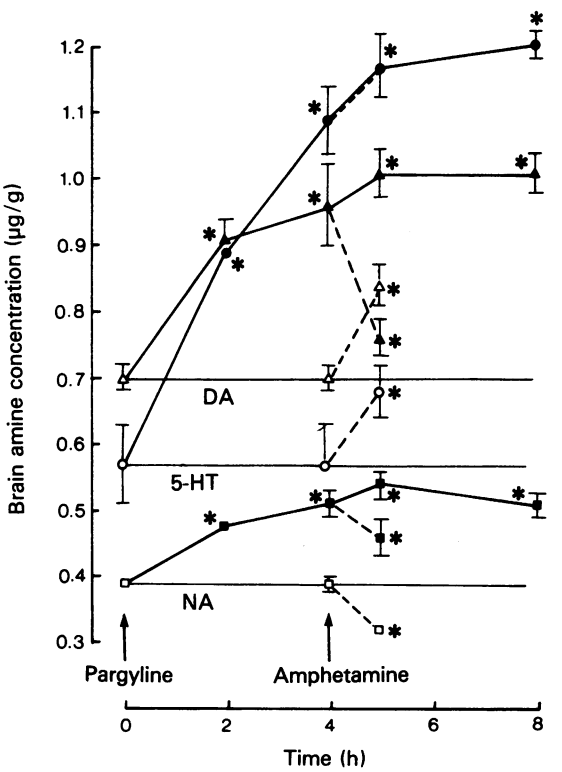

Figure 1 The effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral monoamines of mice treated with pargyline hydrochloride (100 mg/kg). The figure shows the concentration of cerebral noradrenaline (NA, ■——■); dopamine (DA, ▲——▲) and 5-hydroxytryptamine (5-HT, ●——●) of mice treated with pargyline, and the concentration of cerebral noradrenaline (■-----■), dopamine (▲-----▲) and 5-hydroxytryptamine (●----●) of mice treated with amphetamine 4 h after an injection of pargyline. The concentration of the cerebral monoamines of control groups of mice is indicated by open symbols. Each point is the mean of five estimations. Vertical lines indicate the s.e. mean. Difference from the control value, $*P < 0.05$, Student's t test.

before an injection of amphetamine did not appear to influence the effect of the sympathomimetic drug on the concentration of noradrenaline or 5-hydroxytryptamine. However, the effect of amphetamine on the dopamine content was reversed; instead of an increase there was a decrease of 21% in dopamine concentration.

Pargyline caused an increase of 25% in the concentration of brain glycogen. However, there was no significant difference in the extent of the depletion of brain glycogen of animals treated with either amphetamine or pargyline and amphetamine (Table 1). Glycogen was depleted by 23% ($P < 0.002$) in the brains of amphetamine-treated mice and was reduced by 19% ($P < 0.002$) in the brains of animals treated with pargyline and amphetamine.

The body temperature of the mice was reduced by approximately 3.5°C and the hypothermia lasted for 4 h, following an injection of pargyline (Figure 2). The maximum body temperature attained after the administration of amphetamine to pargyline pretreated mice was the same as that achieved by the control mice injected with amphetamine, i.e. a hyperthermia of 2.5 to 3.0°C. The increase in locomotor activity and intensity of the central excitation caused by amphetamine was enhanced and prolonged by pretreating the mice with pargyline. None of the animals died as a result of this treatment.

Desmethylimipramine-amphetamine

Mice in groups of five were injected subcutaneously with an appropriate dose of DMI 15 min before an intraperitoneal injection of amphetamine (5 mg/kg). The mice were killed 45 min after the initial injection. Control groups were injected with DMI, amphetamine or distilled water.

Doses of 1 and 4 mg/kg of DMI did not alter the concentration of cerebral glycogen whereas a small but significant increase in concentration was produced by a dose of 10 mg/kg (Figure 3). Amphetamine-induced glycogenolysis was antagonized by 71% at a dose of 10 mg/kg of DMI. Although DMI (10 mg/kg) caused a depression of locomotor activity, there was no obvious antagonism of the excitation caused by amphetamine.

Amphetamine-amphetamine

The effect of two consecutive doses of amphetamine on the concentration of cerebral glycogen was determined. Mice in groups of five were injected with amphetamine (10 mg/kg) and killed at 30 or 60 min. Mice from a third group were injected with amphetamine at the beginning of the experiment and again at 30 min. This group of animals was killed 60 min after the initial injection.

The greatest depletion of brain glycogen occurred at 30 min (Table 2). Administration of a second dose of amphetamine at this time did not result in further depletion of brain glycogen. Sixty min after the initial injection, the concentration of glycogen did not differ significantly in the brains of mice treated with either a single dose or two doses of amphetamine.

Discussion

Inhibitors of monoamine oxidase (MAO) are known to facilitate the effect of amphetamine on behaviour (Stein, 1964) and to potentiate amphetamine-induced toxicity (Brownlee & Williams, 1963). It has been suggested that the enhanced effect of amphetamine occurs as a result of an increase in the release of catecholamines (Sjoqvist, 1965).

The concentrations of the monoamines (Figure 1) and glycogen (Table 1) of the mouse brain were elevated for at least 4 h after the injection of pargyline. Inhibition of MAO leads to an increase in the concentration of intraneuronal monoamines with little or no increase in the concentration of the extracellular monoamine (Carlsson, Dahlstrom, Fuxe & Lindqvist,

Table 1 The effect of amphetamine on the concentration of brain glycogen of mice treated with pargyline

Treatment	*Dose (mg/kg)*	*Time (h)*	*Brain glycogen concentration* (mg/100 g ± s.e. mean)	*% decrease*
Control	—	—	39.8 ± 1.4	—
Amphetamine	5	0.5	30.5 ± 1.3	23.4*
Pargyline	100	4	49.8 ± 1.0	—
Pargyline + amphetamine	100 5	4 0.5	40.4 ± 1.3	18.9*

Glycogen values show the mean ± s.e. mean for 5 estimations. Difference from the control values, *$P < 0.002$, Student's t test.

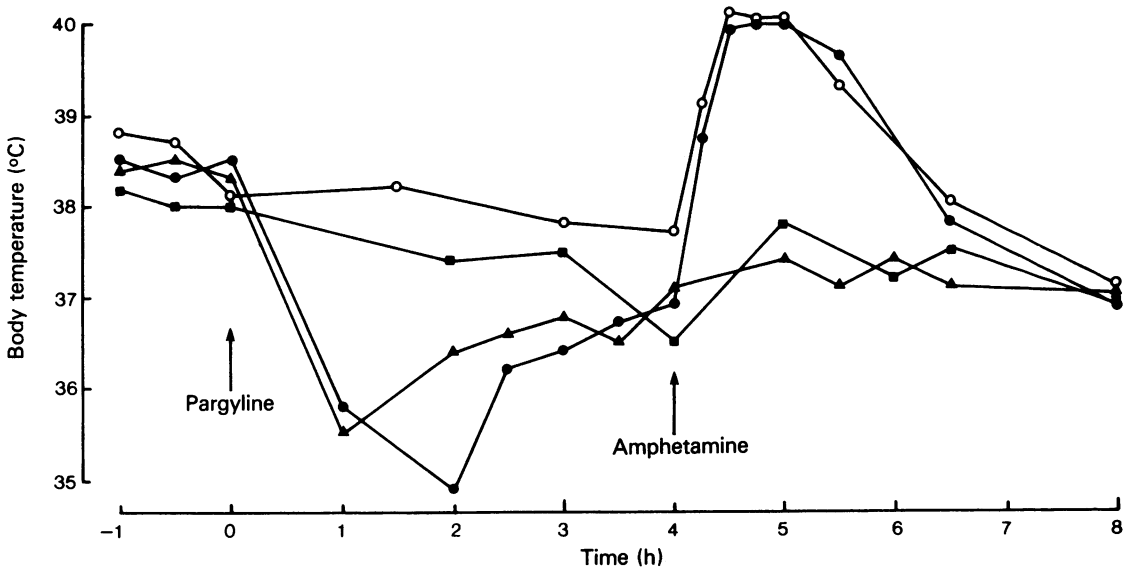

Figure 2 The effect of amphetamine sulphate (5 mg/kg) on the body temperature of mice treated with pargyline hydrochloride (100 mg/kg). Mice in groups of five were injected with pargyline 4 h before the administration of amphetamine (●). Mice in the control group were injected with pargyline (▲), amphetamine (○) or distilled water (■).

1965). Thus the increase in the glycogen content may be due to a reduction in the release of noradrenaline onto central receptor sites, even though the total monoamine content of the brain is increased (Hutchins & Rogers, 1970). However, neither the decrease in noradrenaline content nor the loss of glycogen caused by amphetamine was enhanced when amphetamine was administered to animals pretreated with pargyline. The same peak values in body temperature were achieved also, although the behavioural response of the mice was intensified and prolonged. This finding that inhibition of MAO does not potentiate the effect of amphetamine on noradrenaline or glycogen argues against the possibility of the facilitated effect of amphetamine on behaviour being mediated centrally by an action on noradrenaline release. Brittain, Jack & Spencer (1964) have suggested that the increase in amphetamine toxicity in animals treated with an inhibitor of MAO arises from an enhanced effect of amphetamine on peripheral tissues rather than on the CNS.

It is of interest that amphetamine caused a decrease instead of an increase in the concentration of dopamine, in mice treated with an inhibitor of MAO. If the decrease in dopamine content is due to the release of central dopamine, this may contribute to the facilitated behavioural effect of amphetamine in these animals, although stimulation of dopamine receptors would not therefore appear to promote cerebral glycogenolytic activity.

Table 2 The effect of consecutive doses of amphetamine on the concentration of glycogen in the mouse brain

Treatment	*Dose (mg/kg)*	*Time after initial injection (h)*	*Brain glycogen concentration* (mg/100 g ± s.e. mean)
Control	—	—	41.8 ± 0.5
Amphetamine	10	0.5	28.6 ± 0.4*
Amphetamine	10	1.0	35.5 ± 1.8*
Amphetamine + amphetamine	10 10	1.0	35.8 ± 0.9*

Glycogen values indicate the mean ± s.e. mean for 5 estimations. Difference from the control value, $*P < 0.001$, Student's t test.

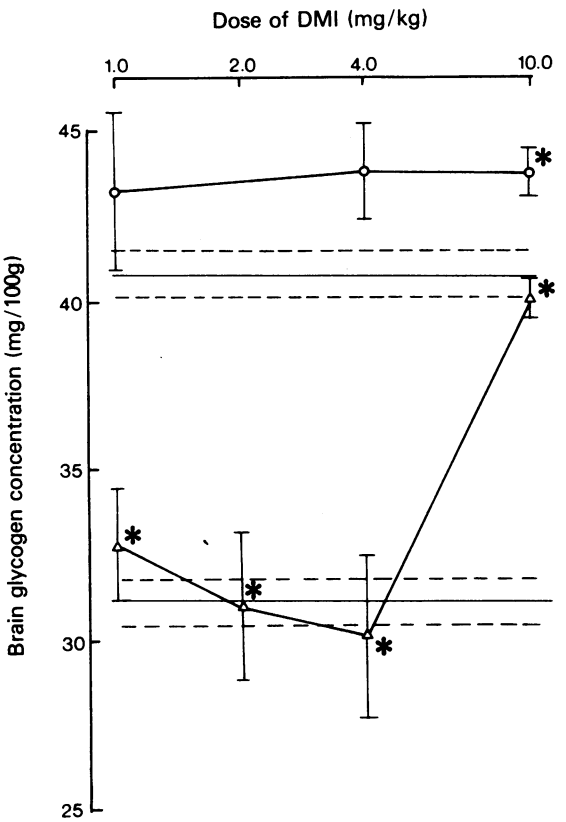

Figure 3 The effect of amphetamine sulphate (5 mg/kg) on the concentration of brain glycogen of mice treated with desmethylimipramine (DMI). Mice in groups of five were injected subcutaneously with DMI initially, distilled water intraperitoneally at 15 min, and then killed at 45 min (○), or they were injected with DMI 15 min before the administration of amphetamine and killed 45 min after the initial injection (△). The horizontal lines indicate the glycogen concentration of mice 30 min after an injection of distilled water (upper line) or amphetamine (lower line). Broken horizontal lines and vertical bars indicate the respective s.e means. Difference from the control value, $*P < 0.05$, Student's t test.

The tricyclic antidepressant drugs such as DMI and imipramine are believed to act mainly by selectively blocking the uptake mechanism of central monoamine neurones (Glowinski & Axelrod, 1964; Carlsson, Fuxe, Hamberger & Lindqvist, 1966). Pretreatment of several species with DMI has been shown to potentiate the central excitatory effects of amphetamine (Carlton, 1961; Stein, 1964; Dolfini, Tansella, Valzelli & Garattini, 1969). The potentiated effects of amphetamine are not related necessarily to inhibition of the reuptake mechanism by DMI, as the antidepressant drugs also inhibit the *in vivo* hydroxylation of amphetamine, thus increasing the amount of unmetabolized amphetamine in the brain (Consolo, Dolfini, Garattini & Valzelli, 1967; Lewander, 1969). However, the mouse does not metabolize amphetamine by hydroxylation and in this species DMI does not increase the concentration of amphetamine in the brain, nor does it potentiate amphetamine-induced excitation (Dolfini *et al.*, 1969). In the mouse DMI also has a much shorter half-life than in the rat (Dingell, Dulser & Gillette, 1964).

The effect of amphetamine on the glycogen content of the brain and on the behaviour of the mice was not potentiated by DMI. These results are in accord with the observations of Dolfini *et al.* (1969). The antagonism of amphetamine-induced glycogenolysis by DMI (10 mg/kg) is possibly due to the adrenoceptor blocking activity of the antidepressant drug (Thoenen, Hurliman & Haefely, 1964). The antagonism of the effect of amphetamine on brain glycogen by β-adrenoceptor blocking drugs has been described previously (Hutchins & Rogers, 1971).

A similar biphasic fluctuation in the concentration of brain glycogen occurred in mice, whether amphetamine was administered as a single dose, or as two doses with a 30 min interval between injections (Table 2). Assuming that amphetamine-like compounds exert similar effects on the adrenergic neurones of the CNS, these results are consistent with the observations of Jonsson, Grobecker & Holtz (1966). They found that β-phenylethylamine depleted the noradrenaline content of the rat brain, but when this drug was injected in two doses, the second dose being given 1 h after the first one, no further decrease of the noradrenaline content took place. These observations may reflect a temporary depletion of the noradrenaline stores available for continued release. On the other hand, if amphetamine exerts a glycogenolytic effect in the brain by a direct action on receptors, a cumulative decrease in glycogen content might be expected to accompany successive doses of the stimulant drug. However, the effect of the second dose of amphetamine may be over-shadowed by the rapid compensatory glycogenesis which appears invariably to follow the initial depletion of brain glycogen.

I wish to thank Dr K.J. Rogers for helpful discussions during the course of this work.

References

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminium oxide trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.* **138,** 360–375.

LE BARON, F.N. (1955). The resynthesis of glycogen by guinea-pig cerebral cortex slices. *Biochem. J.*, **61,** 80–85.

BOGDANSKI, D.F., PLETSCHER, A., BRODIE, B.B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.*, **117,** 82–88.

BRITTAIN, R.T., JACK, D. & SPENCER, P.S.J. (1964). Mechanism of action of monoamine oxidase inhibitors in enhancing amphetamine toxicity. *J. Pharm. Pharmac.*, **16,** 565–567.

BRITTAIN, R.T. & SPENCER, P.S.J. (1964). Measurements of body temperature in conscious small laboratory animals by means of an oesophageal thermocouple. *J. Pharm. Pharmac.* **16,** 497–499.

BROWNLEE, G. & WILLIAMS, G.N. (1963). Potentiation of amphetamine and pethidine by monoamineoxidase inhibitors. *Lancet*, **i,** 669.

CARLSSON, A., DAHLSTROM, A., FUXE, K. & LINDQVIST, M. (1965). Histochemical and biochemical detection of monoamine release from brain neurones. *Life Sci.*, **4,** 809–816.

CARLSSON, A., FUXE, K., HAMBERGER, B. & LINDQVIST, M. (1966). Biochemical and histochemical studies on the effects of imipramine-like drugs and (+)-amphetamine on central and peripheral catecholamine neurons. *Acta. physiol. Scand.*, **67,** 481–497.

CARLSSON, A. & WALDECK, B. (1958). A fluorimetric method for the determination of dopamine (3-hydroxytyramine). *Acta physiol. scand.*, **44,** 293–298.

CARLTON, P.L. (1961). Potentiation of the behavioural effects of amphetamine by imipramine. *Psychopharmacologia*, **2,** 364–376.

CONSOLO, S., DOLFINI, E., GARATTINI, S. & VALZELLI, L. (1967). Desipramine and amphetamine metabolism. *J. Pharm. Pharmac.*, **19,** 253–256.

DINGELL, J.V., SULSER, F. & GILLETTE, J.R. (1964). Species differences in the metabolism of imipramine and desmethylimipramine (DMI). *J. Pharmac. exp. Ther.*, **143,** 14–20.

DOLFINI, E., TANSELLA, M., VALZELLI, L. & GARATTINI, S. (1969). Further studies on the interaction between desipramine and amphetamine. *Eur. J. Pharmac.*, **5,** 185–190.

GLOWINSKI, J. & AXELROD, J. (1964). Inhibition of uptake of tritiated-noradrenaline in the intact rat brain by imipramine and structurally related compounds. *Nature, Lond.*, **204,** 1318–1319.

HUTCHINS, D.A. & ROGERS K.J. (1970). Physiological and drug-induced changes in the glycogen content of mouse brain. *Br. J. Pharmac.*, **39,** 9–25.

HUTCHINS, D.A. & ROGERS K.J. (1971). Effect of receptor blocking drugs on the depletion of brain glycogen by amphetamine. *Br. J. Pharmac.*, **43,** 504–513.

HUTCHINS, D.A. & ROGERS, K.J. (1973). Effect of depletion of cerebral monoamines on the concentration of glycogen and on amphetamine-induced glycogenolysis in the brain. *Br. J. Pharmac.*, **48,** 19–29.

JONSSON, J., GROBECKER, H. & HOLTZ, P. (1966). Effect of β-phenyl-ethylamine on content and subcellular distribution of norepinephrine in rat heart and brain. *Life Sci.*, **5,** 2235–2246.

LEWANDER, T. (1969). Influence of various psychoactive drugs on the *in vivo* metabolism of d-amphetamine in the rat. *Eur. J. Pharmac.* **6,** 38–44.

ROGERS K.J. & HUTCHINS, D.A. (1972). Studies on the relation of chemical structure to glycogenolytic activity in the brain. *Eur. J. Pharmac.*, **20,** 97–103.

SHORE, P.A. & OLIN, J.S. (1958). Identification and chemical assay of norepinephrine in brain and other tissues. *J. Pharmac. exp. Ther.* **122,** 295–300.

SJOQVIST, F. (1965). Psychotropic drugs. Interactive between monoamine oxidase (MAO) inhibitors and other substances. *Proc. R. Soc. Med.* **58,** 967–978.

STEIN, L. (1964). Self-stimulation of the brain and the central stimulant action of amphetamine. *Fedn. Proc.*, **23,** 836–850.

THOENEN, H., HURLIMANN, A. & HAEFELY, W. (1964). Mode of action of imipramine and 5-(3[1]-methylamino propyliden)-dibenzo a,e, cyclohepta (1,3,5) trien hydrochloride (Ro 4-6011), a new antidepressant drug, on peripheral adrenergic mechanisms. *J. Pharmac. exp. Ther.*, **144,** 405–414.

(Received July 24, 1978.)