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Effect of receptor blocking drugs on the depletion of brain glycogen by amphetamine

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Summary

1. Amphetamine sulphate (5 mg/kg), administered intraperitoneally, reduces the concentration of glycogen in the mouse brain by 25–30% after 30 minutes.
2. The effect of several receptor blocking drugs on the amphetamine-induced cerebral glycogenolysis was studied.
3. DL-Propranolol (0·25 mg/kg) and pronethalol (10 mg/kg) antagonized the depletion of brain glycogen by amphetamine.
4. Phentolamine, methysergide, atropine and mepyramine failed to antagonize the amphetamine-induced glycogenolysis.
5. D-Propranolol, chlorpromazine and phenoxybenzamine antagonized the glycogenolytic effect of amphetamine only when administered in sedative doses.
6. It is concluded that amphetamine-induced glycogenolysis in the mouse brain may be mediated through a β-adrenoceptor.

Introduction

The metabolism of glycogen in peripheral tissues is controlled, at least in part, by the action of catecholamines on the adenyl cyclase system (Sutherland & Rall, 1960). As many of the enzymes and cofactors associated with glycogen metabolism occur in the brain it is reasonable to assume a degree of similarity in the metabolism of glycogen in both central and peripheral tissues. The catecholamines noradrenaline and dopamine are believed to function as neurotransmitter substances in the central nervous system (CNS), and since catecholamines in the circulation penetrate the blood-brain barrier only with difficulty it is possible that a change in the concentration of cerebral glycogen may reflect an altered metabolic state of the catecholamines in the CNS.

In a recent study, drugs from several pharmacological classes were investigated for their *in vivo* effect on the concentration of glycogen in the mouse brain (Hutchins & Rogers, 1970). Only amphetamine-like compounds and bemegride-induced convulsions depleted brain glycogen. In view of the evidence that brain noradrenaline is released by convulsions (Breitner, Picchioni & Chin, 1963 ; Maynert & Levi, 1964) and by amphetamine (Glowinski & Axelrod, 1965 ; Smith, 1965) and since stimulation of the CNS by other drugs did not result in glycogen depletion, it was suggested that cerebral glycogenolysis might be stimulated by the release of intraneuronal catecholamines on to extracellular receptor sites, rather than by a general

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increase in the metabolic activity of the CNS (Hutchins & Rogers, 1970). This paper describes experiments in which several drugs classified as receptor blocking agents were examined for their ability to antagonize the glycogenolytic effect of amphetamine in the mouse brain.

Methods

The experiments were performed on male albino mice weighing 20–30 g. The animals were allowed free access to food and water before and during the experiments and the environmental temperature was controlled at 20°–22° C.

Groups of four–five mice were injected subcutaneously with the receptor blocking drug or distilled water 15 min before the administration of D-amphetamine (5 mg/kg) by the intraperitoneal route. The animals were killed 45 min after the initial injection. Control groups of mice were treated with the antagonist or with distilled water.

In order to minimize the effect of circadian fluctuations in the concentration of brain glycogen (Hutchins & Rogers, 1970), the experimental animals were killed within 1 h of the control mice. Experiments were performed in the late evening.

Estimation of brain glycogen

The mice were killed by complete immersion in liquid nitrogen, and the brains were chiselled out of the skull whilst deeply frozen. Each brain was weighed rapidly before crushing on a stainless steel anvil cooled with liquid nitrogen (Stone, 1938). One mouse brain was used for each determination.

Cerebral glycogen was determined using a modification (Hutchins & Rogers, 1970) of the method of Le Baron (1955).

Locomotor activity

Spontaneous locomotor activity was recorded using an activity cage (Ugo Basile, Milan) in which movement of the mice was measured by completion of electronic circuits as the animals moved across the bars on the floor of the cage. Naïve mice were placed in the activity cage for 30 min before injection and the recording of activity. The motor activity of at least thirty mice in groups of three animals was determined for each drug treatment. The mice were injected as described above, and motor activity was recorded for 120 min after the second injection.

Drugs

The drugs used were D-amphetamine sulphate, atropine sulphate, chlorpromazine hydrochloride, mepyramine maleate, methysergide, phenoxybenzamine hydrochloride, phentolamine mesylate, pronethalol hydrochloride, DL-propranolol hydrochloride and D-propranolol hydrochloride.

The drugs were dissolved in distilled water and each drug was administered in a volume of 1·0 ml/100 g body weight. Drug doses are given in terms of the salts where these were used.

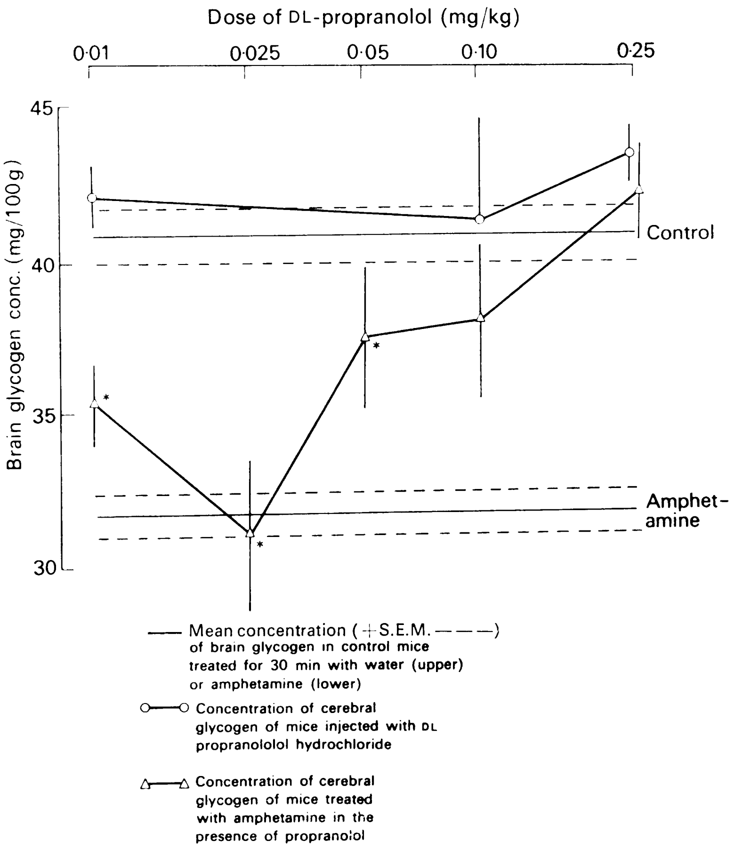

FIG. 1. Effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral glycogen, in mice pretreated with increasing doses of DL-propranolol hydrochloride. The drug dosage regimen is described in **Methods**. Vertical bars indicate the S.E.M. Asterisks indicate a significant difference from the control value ($P<0.05$).

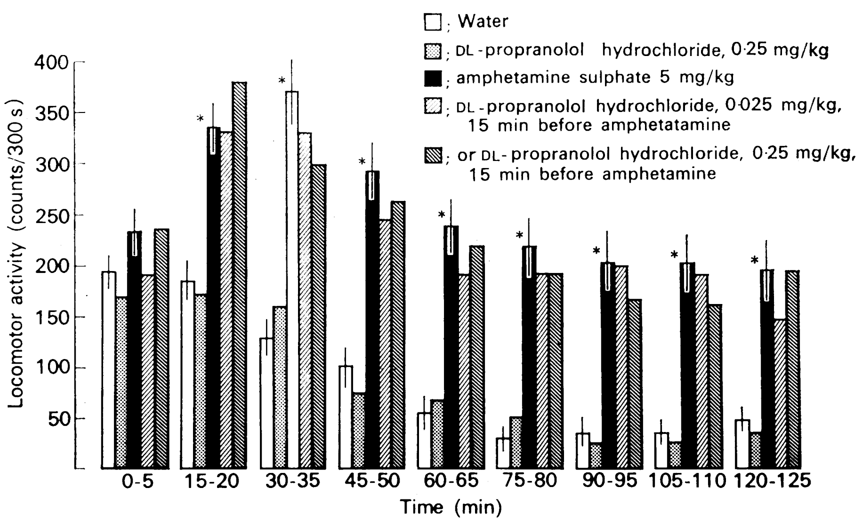

FIG. 2. Effect of amphetamine on the locomotor activity of mice pretreated with DL-propranolol. The mice were injected with the substances shown in the key 15 min before amphetamine. The drug dosage regimen is described in **Methods.** Vertical lines indicate the S.E.M. The S.E.M. of some groups has been omitted for clarity. Asterisks indicate a significant difference from the control value ($P<0.05$).

Results

DL-*Propranolol*

DL-Propranolol was a potent antagonist of amphetamine-induced glycogenolysis (Fig. 1). Total inhibition of glycogenolysis was achieved at 0·25 mg/kg, a dose of DL-propranolol which did not alter the glycogen concentration *per se* and did not inhibit the locomotor activity of control mice or the increased activity of mice treated with amphetamine (Fig. 2).

D-*Propranolol*

The dextro isomer of propranolol was 80 times less potent than the racemate in its inhibitory effect on amphetamine-induced glycogenolysis. Doses of D-propranolol (5 and 20 mg/kg) caused sedation and increased the concentration of brain glycogen (Fig. 3). The higher dose antagonized the glycogenolytic action of amphetamine.

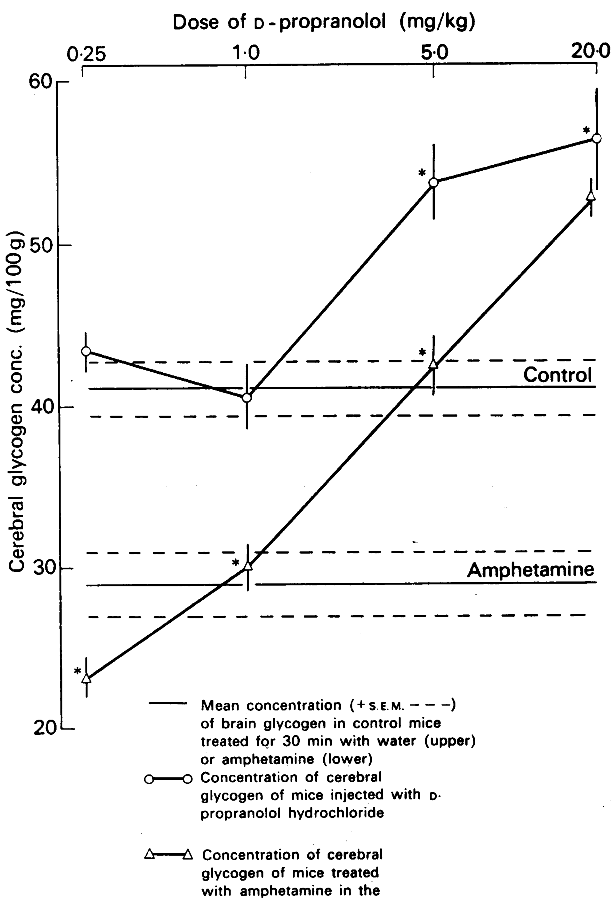

FIG. 3. Effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral glycogen, in mice pretreated with increasing doses of D-propranolol hydrochloride. The drug dosage regimen is described in **Methods**. Vertical bars indicate the S.E.M. Asterisks indicate a significant difference from the control value ($P<0{\cdot}05$).

Pronethalol

The β-adrenoceptor blocking agent pronethalol, at the smallest doses administered (0·25 and 1·0 mg/kg), stimulated glycogenolysis in the mouse brain (Fig. 4) and increased locomotor activity. Potentiation of the amphetamine-induced glycogenolysis was observed after these doses of pronethalol. The behaviour of the mice was unaffected by pronethalol administered in doses of 2·5–10·0 mg/kg, but the concentration of cerebral glycogen increased with logarithmic increase in dose. Total inhibition of amphetamine-induced glycogenolysis was achieved after a dose of pronethalol (10·0 mg/kg).

Phentolamine

Except for some ptosis and slight depression of locomotor activity at doses greater than 40 mg/kg, phentolamine had little effect on the behaviour of the mice.

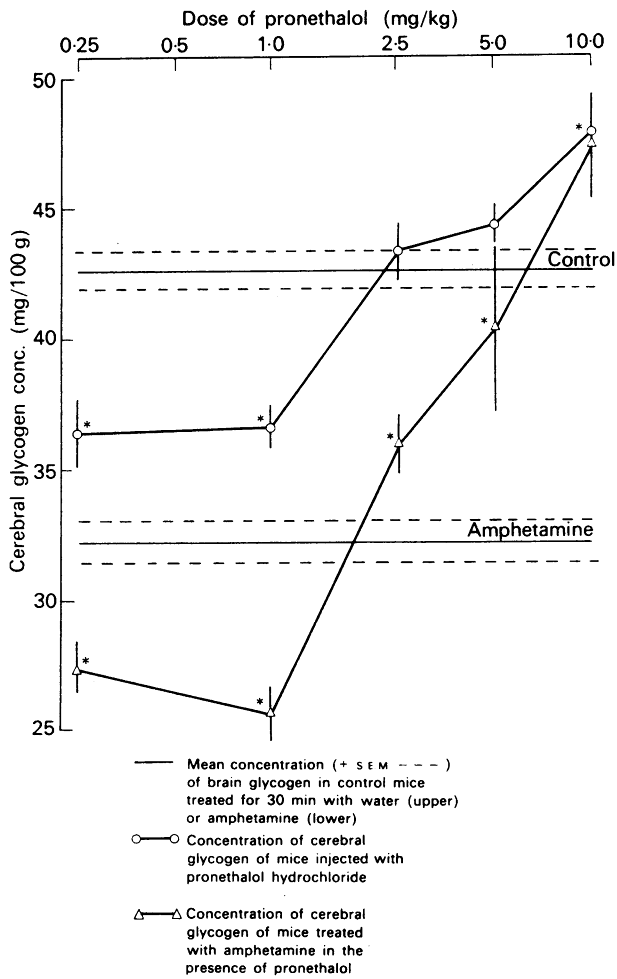

FIG. 4. Effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral glycogen, in mice pretreated with increasing doses of pronethalol hydrochloride. The drug dosage regimen is described in **Methods.** Vertical bars indicate the S.E.M. Asterisks indicate a significant difference from the control value ($P<0·05$).

A significant decrease in cerebral glycogen content ($P<0.05$) occurred after the injection of 40 mg/kg and 160 mg/kg of this drug (Fig. 5). Phentolamine appeared to cause no major modification of the behavioural excitation induced by amphetamine, and unlike the β-adrenoceptor blocking drugs, increasing doses of phentolamine did not antagonize the glycogenolytic effect of amphetamine.

Methysergide

Methysergide is a specific antagonist at 5-hydroxytryptamine receptors. Methysergide (less than 10 mg/kg) caused an initial depression of locomotor activity lasting approximately 10 min, after which time the behaviour of the drug-injected mice was indistinguishable from that of mice treated with distilled water. The glycogen content of the mouse brain was not altered significantly by methysergide and this drug failed to antagonize the depletion of brain glycogen caused by amphetamine (Fig. 6).

Phenoxybenzamine, chlorpromazine, atropine and mepyramine

Only sedative doses of the α-adrenoceptor blocking agent phenoxybenzamine (50 mg/kg) and chlorpromazine (5 mg/kg) antagonized amphetamine-induced glycogenolysis (Fig. 7). Non-sedative doses of these drugs failed to inhibit the depletion of glycogen by amphetamine. Atropine, a muscarinic receptor blocking drug and

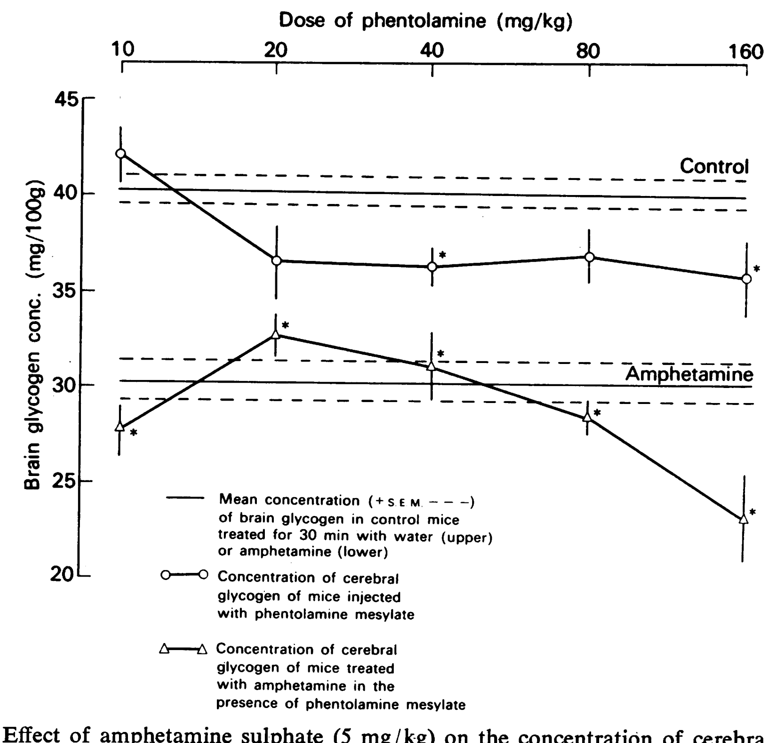

FIG. 5. Effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral glycogen, in mice pretreated with increasing doses of phentolamine mesylate. The drug dosage regimen is described in **Methods**. Vertical bars indicate S.E.M. Asterisks indicate a significant difference from the control value ($P<0.05$).

mepyramine, an antihistamine did not antagonize amphetamine-induced glycogenolysis.

Discussion

Catecholamines stimulate glycogen breakdown in peripheral tissues, through activation of the adenyl cyclase system. However, considerable tissue and species variation is shown with respect to the adrenoceptor mediating this glycogenolytic effect of the catecholamines. Whereas glycogenolysis in the liver of the cat (Ellis & Eusebi, 1965) and dog (Mayer, Moran & Fain, 1961 ; Murad, Chi, Rall & Sutherland, 1962) appear to be mediated by the β-adrenoceptor, this response may be subserved by the α-adrenoceptor in man (Antonis, Clark, Hodge, Molony & Pilkington, 1967) and rat (Fleming & Kenny, 1964 ; Adnitt, 1969).

This investigation has shown that the β-adrenoceptor blocking drugs, DL-propranolol and pronethalol antagonized the depletion of brain glycogen by amphetamine at doses of the antagonist drugs that did not affect behaviour. Pronethalol

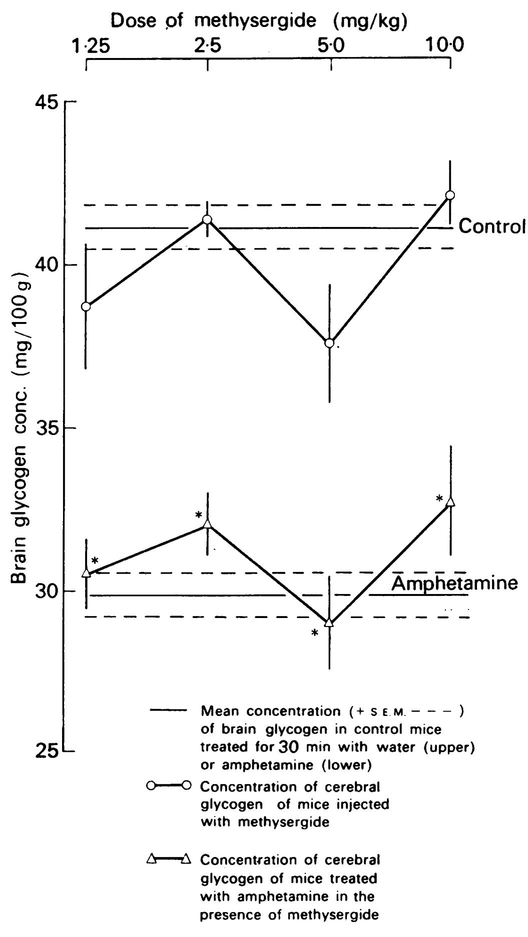

FIG. 6. Effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral glycogen, in mice pretreated with increasing doses of methysergide. The drug dosage regimen is described in **Methods.** Vertical bars indicate the S.E.M. Asterisks indicate a significant difference from the control value ($P<0·05$).

was approximately one-fortieth as active as DL-propranolol. Pronethalol has a low level of β-adrenoceptor agonist activity (Goodman & Gilman, 1965), and in this context the depletion of brain glycogen caused by low doses of pronethalol is consistent with β-adrenoceptor stimulation. With increasing doses, the β-adrenoceptor blocking activity eventually overrides the sympathomimetic effect, and at still higher doses the glycogenolysis caused by amphetamine is inhibited.

The results showing that racemic propranolol blocks amphetamine-induced glycogenolysis, supports and extends the observations of Estler & Ammon (1967), who found that a single dose of propranolol, which by itself increased brain glycogen, inhibited the reduction in glycogen caused by methamphetamine. It is unlikely that the low dose of DL-propranolol (0·25 mg/kg), used in the present study to antagonize the effect of amphetamine on brain glycogen (Fig. 1), exerted a central depressant action, as spontaneous locomotor activity and amphetamine-induced stimulation of activity were not influenced by this dose of DL-propranolol. In order to exclude membrane stabilization as a possible mechanism of the inhibitory action of DL-propranolol on amphetamine-induced glycogenolysis, the effect of D-propranolol was investigated. The dextro isomer of propranolol has membrane stabilizing activity but is virtually devoid of β-adrenoceptor blocking properties (Howe & Shanks, 1966 ; Barett & Cullum, 1968). D-Propranolol was 80 times less active than the racemate in blocking the glycogenolytic action of amphetamine. A similar ratio of activities of the two isomers on the cardiovascular system was ob-

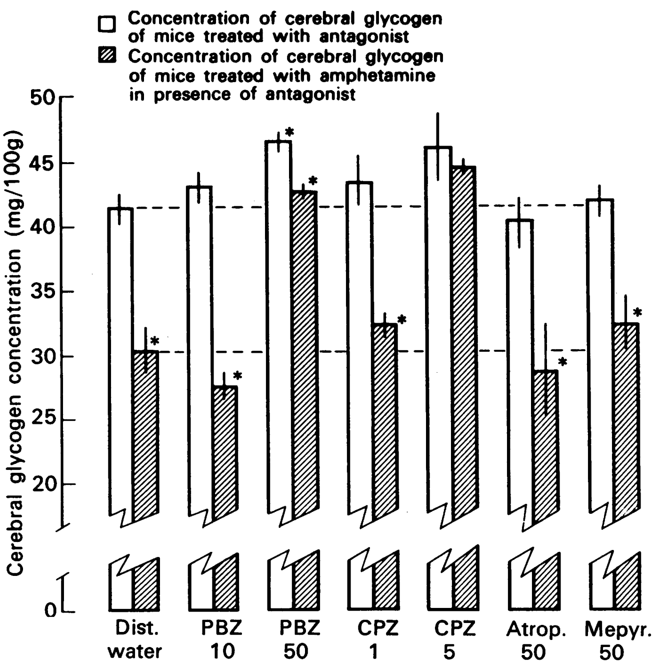

FIG. 7. Effect of amphetamine sulphate (5 mg/kg) on the concentration of cerebral glycogen in mice pretreated with various receptor blocking drugs. The drug dosage regimen is described in **Methods**. Drugs used: distilled water (dist. water); phenoxybenzamine hydrochloride, 10 mg/kg (PBZ, 10) and 50 mg/kg (PBZ, 50); chlorpromazine hydrochloride, 1 mg/kg (CPZ, 1) and 5 mg/kg (CPZ, 5); atropine sulphate, 50 mg/kg (Atrop, 50); and mepyramine maleate, 50 mg/kg (Mepyr, 50). Vertical bars indicate the S.E.M. Asterisks indicate a significant difference from the control value ($P<0·05$).

served by Barrett (1969). It is concluded that the antagonism of amphetamine-induced glycogenolysis by racemic propranolol (0·25 mg/kg) is due to specific blockade of β-adrenoceptors and is not a result of membrane stabilization.

Phenoxybenzamine antagonized amphetamine-induced glycogenolysis only at a dose that caused sedation (50 mg/kg). High doses of phenoxybenzamine have central depressant effects that are not related to the α-adrenoceptor blocking ability of this drug (Goodman & Gilman, 1965). Thus the small increase in glycogen after phenoxybenzamine (50 mg/kg) and inhibition of the stimulant effects of amphetamine on motor activity and glycogenolysis may be the result of a non-specific central depression.

Neither the behavioural excitation nor the stimulation of glycogenolysis caused by amphetamine were antagonized in mice by chlorpromazine (1 mg/kg). A higher dose of chlorpromazine (5 mg/kg) which of itself caused behavioural sedation, inhibited the excitation and locomotor stimulant effect of amphetamine and antagonized the depletion of glycogen. Neuroleptic drugs such as chlorpromazine and haloperidol block central dopamine receptors (Carlsson & Lindqvist, 1963; Nyback & Sedvall, 1968), and α-adrenoceptors in peripheral tissues (Holzbauer & Vogt, 1954; Moran & Butler, 1956) and in the CNS (Dell, 1960; Ilyutchenok, 1968). If the glycogenolytic effect of amphetamine is mediated by the stimulation of dopamine or α-adrenoceptors, then the block of these receptors by chlorpromazine may be responsible for the antagonism of the pharmacological effects exerted by amphetamine. However, the pharmacological action of chlorpromazine is far from being limited to the blocking of dopamine receptors or adrenoceptors. Chlorpromazine also has antiacetylcholine, antihistamine and anti-5-hydroxytryptamine properties, but since atropine mepyramine and methysergide failed to antagonize amphetamine-induced glycogenolysis it would appear unlikely that these receptor blocking actions of chlorpromazine contribute to the inhibition of the amphetamine effects.

Amphetamine exerts a complex action on catecholamine containing neurones (see Schildkraut & Kety, 1967). It releases cerebral catecholamines and also inhibits their reuptake into central neurones. Amphetamine may exert also a direct action on adrenoceptors in the brain. It is possible, therefore, that the depletion of brain glycogen by amphetamine may be mediated by a direct action at central adrenoceptors or by an indirect action resulting from an increase in the concentration of extraneuronal transmitter. These studies with various receptor blocking drugs suggest that amphetamine-induced glycogenolysis in mouse brain is mediated through the β-adrenoceptor.

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REFERENCES

ADNITT, P. I. (1969). Hepatic glycogen and blood glucose control. *Biochem. Pharmac.*, **18**, 2599–2604.

ANTONIS, A., CLARK, M. L., HODGE, R. L., MOLONY, M. & PILKINGTON, T. R. (1967). Receptor mechanisms in the hyperglycaemic response to adrenaline in man. *Lancet*, **1**, 1135–1137.

BARRETT, A. M. (1969). A comparison of the effects of (±)-propranolol and (+)-propranolol in anaesthetized dogs; β-receptor blocking and haemodynamic action. *J. Pharm. Pharmac.*, **21**, 241–247.

BARRETT, A. M. & CULLUM, V. A. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43–55.

BREITNER, C., PICCHIONI, A. & CHIN, L. (1963). Neurohormone levels in brain after CNS stimulation including electrotherapy. *J. Neuropsychiat.*, **5**, 153–158.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmac. tox.*, **20**, 140–144.

DELL, P. (1960). Intervention of an adrenergic mechanism during brain stem reticular activation. In: *Adrenergic Mechanisms*, ed. Vane, J. R. & Wolstenholme, G. E. W. Ciba Foundation Symposium.

ELLIS, S. & EUSEBI, A. J. (1965). Dissociation of epinephrine-induced hyperkalaemia and hyperglycaemia by adrenergic blocking drugs and theophylline: Role of cyclic 3′5′-AMP. *Fedn Proc.*, **24**, 151.

ESTLER, C. J. & AMMON, H. P. T. (1967). The influence of propranolol on the methamphetamine-induced changes of cerebral function and metabolism. *J. Neurochem.*, **14**, 799–806.

FLEMING, W. W. & KENNY, A. D. (1964). The effect of fasting on the hyperglycaemic responses to catecholamines in rats. *Br. J. Pharmac.*, *Pharmac.*, **22**, 267–274.

GLOWINSKI, J. & AXELROD, J. (1965). Effect of drugs on the uptake, release and metabolism of H^3-norepinephrine in the rat brain. *J. Pharmac. exp. Ther.*, **148**, 43–49.

GOODMAN, L. S. & GILMAN, A. (1965). *The Pharmacological Basis of Therapeutics*. New York: MacMillan.

HOLZBAUER, M. & VOGT, M. (1954). The action of chlorpromazine on diencephalic sympathetic activity and on the release of adrenocorticotrophic hormones. *Br. J. Pharmac.*, **9**, 402–407.

HOWE, R. & SHANKS, R. G. (1966). Optical isomers of propranolol. *Nature, Lond.*, **210**, 1336–1338.

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.

ILYUTCHENOK, R. Y. (1968). The central mechanisms of phenothiazine compounds. *Agressologie*, **9**, 365–371.

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

MAYER, S., MORAN, N. C. & FAIN, J. (1961). The effect of adrenergic blocking agents on some metabolic actions of catecholamines. *J. Pharmac. exp. Ther.*, **134**, 18–27.

MAYNERT, E. W. & LEVI, R. (1964). Stress induced release of brain norepinephrine and its inhibition by drugs. *J. Pharmac. exp. Ther.*, **413**, 90–95.

MORAN, N. C. & BUTLER, W. M. (1956). The pharmacological properties of chlorpromazine sulfoxide, a major metabolite of chlorpromazine. A comparison with chlorpromazine. *J. Pharmac. exp. Ther.*, **118**, 328–337.

MURAD, F., CHI, Y.-M., RALL, T. W. & SUTHERLAND, E. W. (1962). Adenyl cyclase III. The effect of catecholamines and choline esters on the formation of adenosine 3′5′-phosphate by preparations from cardiac muscle and liver. *J. biol. Chem.*, **237**, 1233–1238.

NYBACK, H. & SEDVALL, G. (1968). Effect of chlorpromazine on accumulation and disappearance of catecholamines formed from tyrosine-C^{14} in brain. *J. Pharmac. exp. Ther.*, **162**, 294–301.

SCHILDKRAUT, J. J. & KETY, S. S. (1967). Biogenic amines and emotion. *Science, N.Y.*, **156**, 21–30.

SMITH, C. B. (1965). Effect of d-amphetamine upon brain amine content and locomotor activity of mice. *J. Pharmac. exp. Ther.*, **147**, 96–102.

STONE, W. E. (1938). The effects of anaesthetics and of convulsants on the lactic acid content of the brain. *Biochem. J.*, **32**, 1908–1918.

SUTHERLAND, E. W. & RALL, T. W. (1960). The relation of adenosine 3′5′-phosphate and phosphorylase to the actions of catecholamines and other hormones. *Pharmac. Rev.*, **12**, 265–299.

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