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STUDIES ON THE RELATION OF CHEMICAL STRUCTURE TO GLYCOGENOLYTIC ACTIVITY IN THE BRAIN

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The structure–activity relation (SAR) of β-phenylethylamine analogues and other drugs has been studied, in order to determine the molecular requirements for cerebral glycogenolytic activity in the mouse.

The results suggest that for extensive cerebral glycogenolytic activity a β-phenylethylamine nucleus is necessary. Maximum stimulation of glycogenolysis occurred with a cyclopropyl group replacing carbon atoms 1 and 2 (tranylcypromine) or a methyl group on carbon atom 1 (amphetamine) of the alkylamine chain. There was no difference in the potency of the d- and d,l-isomers of amphetamine. Almost total loss of activity occurred if a substitution was made on the benzene ring, or the isopropyl chain of amphetamine. Adrenaline and noradrenaline, but not 5-hydroxytryptamine, increased the concentration of glycogen. Of the other drugs, only relatively large doses of LSD and methysergide caused a decrease in the content of glycogen. Morphine, nicotine and phencyclidine, in subconvulsant doses, did not deplete brain glycogen.

Brain glycogen Amphetamine β-Phenylethylamine analogues Structure–activity relation

1. INTRODUCTION

The concentration of cerebral glycogen in mice is depleted after bemegride-induced convulsions or after treatment with the β-phenylethylamine derivatives amphetamine, tranylcypromine or fencamfamin, whereas cocaine, caffeine and morphine which are not related chemically to β-phenylethylamine do not deplete brain glycogen (Hutchins and Rogers, 1970). It has been shown also, that low doses of propranolol inhibit amphetamine-induced glycogenolysis without antagonising the central stimulant action of amphetamine (Hutchins and Rogers, 1971). These results suggest that increased cerebral glycogenolysis is not an essential concomitant of the central excitation and increased locomotor activity caused by these drugs. A further investigation has been made therefore, to examine the structure–activity relation (SAR) of β-phenylethylamine analogues and other drugs in order to determine the molecular requirements for cerebral glycogenolytic activity.

The SAR of many sympathomimetic drugs has been studied in peripheral tissues but similar studies of the CNS are restricted, not only by considerable difficulties of experimental technique but also by the inability of polar compounds such as catecholamines to cross the blood–brain barrier. For the purposes of the present study d-amphetamine was used as the standard for comparison of the SAR with other β-phenylethylamine analogues. The β-phenylethylamine derivatives used have been classified according to the substitution of chemical groupings on three moieties of the amphetamine molecule: (i) the benzene ring; (ii) the isopropyl group in which a chain of 2 carbon atoms joins the benzene ring to the nitrogen atom; and (iii) the primary amine group. The glycogenolytic activity of other central stimulant drugs and hallucinogenic agents has been investigated also.

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2. MATERIALS AND METHODS

The experiments were performed on male albino mice weighing between 20 and 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 mice were injected with different doses of the test drug and were killed 30 min later. The concentration of glycogen in the brains of the mice was determined, and where appropriate, log dose–response curves were constructed for each of the drugs. The relative glycogenolytic potency of each drug and a statistical analysis of results are shown in table 2.

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

The drugs were dissolved in distilled water and each drug was administered in a volume of 1 ml/100 g body weight. Many of the compounds used were not available resolved into their optical isomers, so unless otherwise stated these drugs were administered as racemic mixtures.

2.1. *Estimation of brain glycogen*

The mice were killed by complete 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 (Stone, 1938). The brain of 1 mouse was used for each determination.

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

3. RESULTS

In a preliminary experiment the time course of the effect of d-amphetamine on cerebral glycogen was determined over a period of 120 min. Maximum depletion of glycogen occurred at 20 min (fig. 1) but analysis of variance showed that there was no significant difference ($F = 1.26$) in the decrease in the concentration of cerebral glycogen at 10, 20 or 30 min after amphetamine injection. In subsequent experiments the effect of the β-phenylethylamine derivatives on the content of cerebral glycogen was determined in mice killed 30 min after injection.

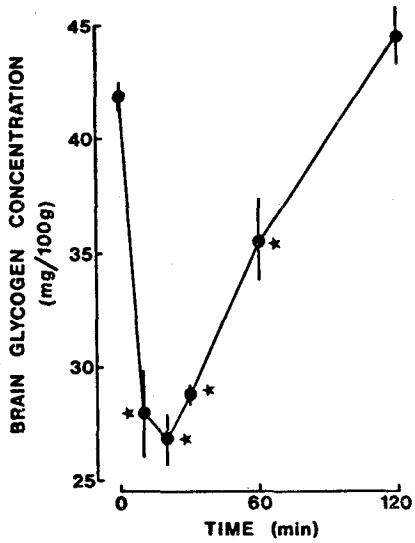

Fig. 1. Time course of the effect of d-amphetamine (25 μmole/kg, i.p.) on the concentration of brain glycogen. Vertical lines indicate the S.E.M. Difference from the control values: * $p < 0.001$, Student's t-test.

3.1. *Stereoisomers of amphetamine*

In order to determine the possible influence of the stereostructure of these compounds on glycogenolytic activity, the cerebral glycogenolytic activities of the d- and d,l-isomers of the standard molecule, amphetamine, were compared over a dose range of 2.5–20 mg/kg. Parallel log dose–response curves, which showed no difference in the potency of the 2 stereoisomers, were obtained. Thus there appears to be no specific stereochemical requirement for the increase in cerebral glycogenolytic activity caused by amphetamine.

3.2. *Glycogenolytic activity of amphetamine analogues*

3.2.1. Replacement of, or substitution on the benzene ring of the amphetamine molecule

The drugs used were 2-aminoheptane, hydroxyamphetamine, alpha-methyltryptamine and norfenflu-

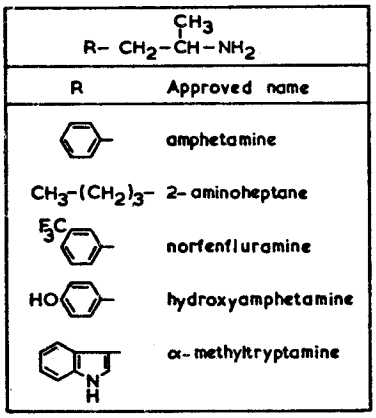

Fig. 2. Chemical structures of amphetamine derivatives. (i) Substitution on the benzene ring.

ramine (fig. 2). Substitution on the benzene ring by CF_3 in the meta position (norfenfluramine) or OH in the para position (hydroxyamphetamine) resulted in 78% and 94% loss of activity respectively. Replacement of the aromatic ring with an aliphatic chain (2-aminoheptane) led to a 31% reduction, but if the benzene ring was replaced with an indole group (α-methyltryptamine) there was a 95% loss of activity. The dose–response curves, determined from derivatives with a substitution on the benzene ring, are illustrated in fig. 3. These are representative of the curves obtained for most of the β-phenylethylamine analogues.

3.2.2. Substitution on the carbon chain

The drugs used were α-phenylethylamine, β-phenylethylamine, phenylpropanolamine and tranylcypromine (fig. 4). Cyclisation of the α- and β-carbon atoms of the amphetamine molecule forms 2-phenylcyclopropylamine (tranylcypromine). This was the only compound that was found to be a more active glycogenolytic agent than amphetamine, with 2.1 times the activity of the latter drug. Hydroxylation of the β-carbon atom (phenylpropanolamine) or demethylation of the α-carbon atom (β-phenylethylamine) reduces glycogenolysis by 84–92%. Reduction of the carbon chain length between the aromatic ring and the amino nitrogen, as shown by α-phenylethylamine, resulted in almost total loss of activity. A maximum reduction of 12.2% ($p < 0.05$) occurred after subconvulsive doses of this drug.

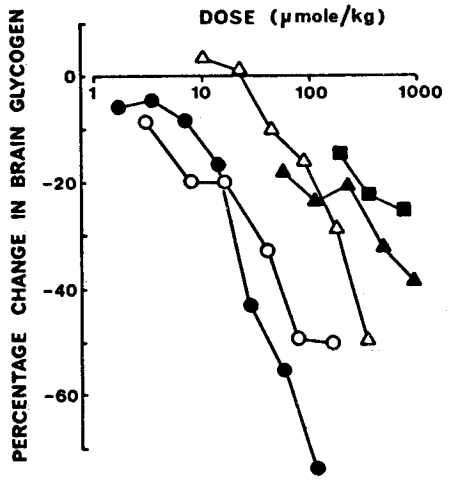

Fig. 3. Dose–response curves for the effect of amphetamine derivatives on the concentration of brain glycogen. (i) Substitution on the benzene ring. Groups of 4 mice were injected i.p. with d-amphetamine (●——●), 2-aminoheptane (○——○), norfenfluramine (△——△), hydroxyamphetamine (▲——▲) or α-methyltryptamine (■——■) and were killed 30 min later. Decreases in the concentration of glycogen greater than 12% were different from the control values: $p <$ 0.05, Student's t-test.

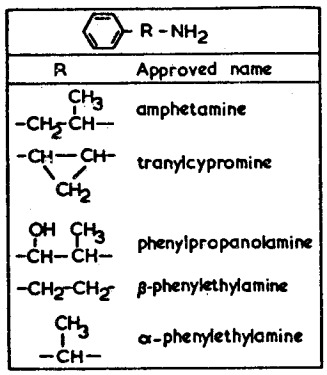

Fig. 4. Chemical structures of amphetamine derivatives. (ii) Substitution on the isopropyl group.

3.2.3. Substitution of the amino group

Methamphetamine is an N-methyl substitution product of amphetamine, and is thus a secondary amine. Benzphetamine is an N-benzyl-N-methyl substituent, and therefore a tertiary amine (fig. 5). d-Methamphetamine, which has the less bulky N-substituent, retained 71% of the glycogenolytic activity

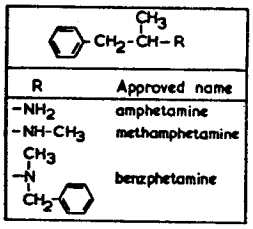

Fig. 5. Chemical structures of amphetamine derivatives. (iii) Substitution on the amino group.

of amphetamine whereas benzphetamine had only 13% of the activity of this drug.

3.2.4. Multiple substitution

Substitution on more than one moiety of the amphetamine molecule (fig. 6) resulted in a loss of glycogenolytic activity that varied from 60% (fencamfamin and p-chloromethamphetamine) to 95% (ethyltryptamine and ephedrine). Mescaline and fenfluramine produced a significant reduction of the glycogen concentration, but neither of these drugs depleted the glycogen by more than 25% at sub-convulsive doses.

The catecholamines noradrenaline and adrenaline not only failed to deplete brain glycogen but caused a highly significant increase in concentration (table 1). This increase was maintained for at least 60 min. In contrast 5-hydroxytryptamine did not alter the concentration of glycogen.

3.3. *Analysis of results*

A statistical analysis of the results is presented in table 2. With few exceptions the log dose–response curve for each drug had a significant regression and was parallel to the standard curve determined for d-amphetamine. The regression coefficients were

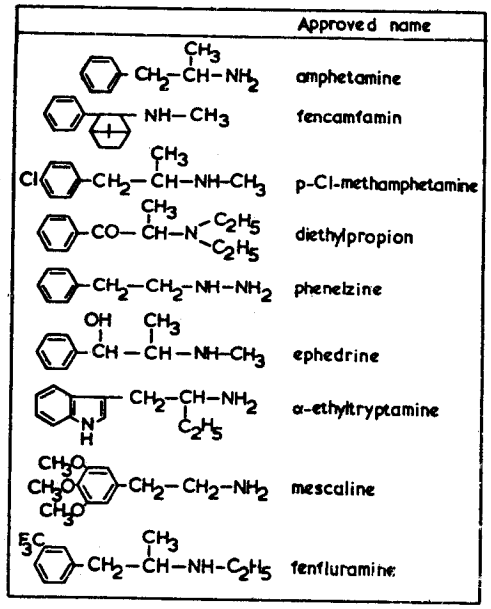

Fig. 6. Chemical structures of amphetamine derivatives. (iv) Multiple substitution.

found to be significantly different from zero for all of the drugs except ephedrine, mescaline, fenfluramine and α-phenylethylamine. Only the regression coefficients for d-methamphetamine and α-methyltryptamine were significantly different from that of d-amphetamine ($p < 0.05$). The lack of parallelism of the log dose–response curves for d-methamphetamine and α-methyltryptamine, therefore, prevented a true comparison of the glycogenolytic potencies of these drugs with that of d-amphetamine. The regression analyses were carried out according to Burn et al. (1960).

Table 1

The effect of noradrenaline, adrenaline and 5-hydroxytryptamine on the concentration of brain glycogen. Glycogen values are means ± S.E.M. Difference from the control value: * $p < 0.001$, Student's t-test.

Treatment	Dose (mg/kg, i.p.)	Number of estimations	Cerebral glycogen concentration (mg/100 g brain)	
			30 min	60 min
–	–	9	42.9 ± 1.1	–
Noradrenaline	1.0	5	59.0 ± 1.5 *	62.4 ± 1.1 *
Adrenaline	1.0	5	59.0 ± 2.2 *	64.3 ± 1.5 *
5-Hydroxytryptamine	10.0	4	44.3 ± 1.2	45.2 ± 0.8

Table 2

Relative glycogenolytic potencies of amphetamine analogues. Mescaline, fenfluramine and α-phenylethylamine decreased the concentration of brain glycogen ($p < 0.05$) by less than 25% of the control value when administered in subconvulsant doses.

Drug	Regression coefficient b ± S.E.M.	Significance of regression $p <$	Parallelism * $p <$	Relative potency ± S.E.M.
Tranylcypromine	−16.4 ± 2.3	0.001	–	209 ± 24
d-Amphetamine	−23.0 ± 3.3	0.001	–	100
Methamphetamine	−51.0 ± 7.6	0.001	0.05	(71 ± 6)
2-Aminoheptane	−18.0 ± 3.3	0.001	–	69 ± 11
Fencamfamin	−15.8 ± 1.9	0.001	–	39 ± 5
p-Cl-methamphetamine	−31.4 ± 3.7	0.001	–	34 ± 4
Norfenfluramine	−16.3 ± 2.0	0.001	–	22 ± 3
β-Phenylethylamine	−26.3 ± 4.3	0.001	–	16 ± 1
Diethylpropion	−32.0 ± 7.0	0.01	–	13 ± 2
Benzphetamine	−35.8 ± 7.5	0.01	–	13 ± 1
Phenelzine	−29.6 ± 4.4	0.001	–	8 ± 1
Phenylpropanolamine	−14.4 ± 3.5	0.01	–	8 ± 1
Ephedrine	−15.7 ± 12.5	–	–	(7 ± 1)
p-Hydroxyamphetamine	−13.0 ± 2.0	0.001	–	6 ± 0.4
α-Ethyltryptamine	−17.2 ± 2.8	0.001	–	5 ± 1
α-Methyltryptamine	−10.2 ± 4.5	0.05	0.05	(5 ± 1)

* Parallelism with respect to d-amphetamine.

3.4. *Miscellaneous drugs*

The glycogenolytic activity of some drugs that possess central stimulant or hallucinogenic properties was determined. Morphine, LSD and methysergide contain a β-phenylethylamine nucleus within their molecular structures (fig. 7). Nicotine and phencyclidine contain no such structure.

Of the drugs that caused stimulation in the mouse, LSD and methysergide depleted brain glycogen (table 3), whereas nicotine and morphine at subconvulsive doses, were without effect. Phencyclidine was the only one of these drugs tested that increased the glycogen concentration.

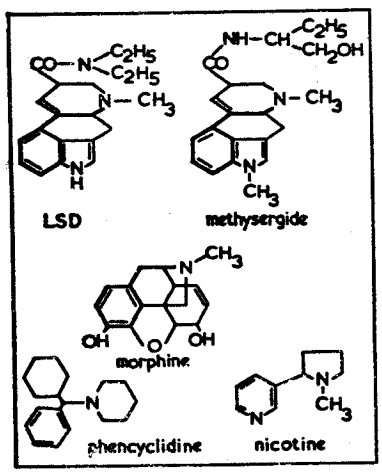

Fig. 7. Chemical structures of some other drugs tested.

Table 3

The effect of miscellaneous drugs on the concentration of brain glycogen. The mice were killed 30 min after drug injection. Glycogen values are means ± S.E.M. The number of estimations is indicated in parentheses. Difference from the control value: * $p < 0.05$, ** $p < 0.001$, Student's *t*-test.

Drug	Dose (mg/kg, i.p.)	Glycogen concentration (mg/100 g)
Control	–	40.4 ± 1.6 (13)
Morphine	50	37.7 ± 1.3 (4)
Morphine	100	41.4 ± 1.0 (4)
Morphine	200	38.8 ± 2.4 (4)
Morphine	400	36.7 ± 0.9 (3)
Control	–	41.9 ± 0.8 (14)
LSD	2.0	38.3 ± 1.2 (8) *
Methysergide	20	36.0 ± 0.6 (4) **
Methysergide	40	33.4 ± 1.4 (4) **
Control	–	40.5 ± 1.0 (8)
Nicotine	10	39.6 ± 1.8 (4)
Phencyclidine	25	47.3 ± 2.3 (4) *

4. DISCUSSION

The role of the peripheral sympathetic nervous system in the regulation of a metabolic function, such as glycogenolysis, may be studied by stimulating the activity of the system, for example, by drug administration or electrostimulation, and comparing the effect with that obtained by the administration of a known quantity of noradrenaline or adrenaline. Projection of this procedure to the CNS is complicated by the difficulty with which exogenous catecholamines cross the blood–brain barrier (Weil-Malherbe et al., 1961). However, the adoption of an alternative substance capable of stimulating glycogenolysis in the CNS, for example amphetamine, might provide a means of studying indirectly the SAR requirements for stimulation of central catecholamine receptors.

In the present study tranylcypromine was the only drug that was found to be more potent than amphetamine. Tranylcypromine contains a cyclopropyl group in the aliphatic side chain. Any other form of substitution on the carbon chain resulted in a loss of at least 60% of activity, which suggests that the isopropyl moiety makes a major contribution to the glycogenolytic activity. An unsubstituted benzene ring also appears to be an important requirement. Substitution on the aromatic ring or replacement of the ring with an indole group reduced activity by approximately 90%. However, 2-aminoheptane was only 30% less active than amphetamine, possibly because the aliphatic chain of the 2-aminoheptane molecule is capable of assuming a ring-like structure. The size of the substituent group on the terminal nitrogen atom also appeared to influence the degree of activity. Benzphetamine, the molecule with the bulkiest group was the least active glycogenolytic agent in this group. Substitution on more than one moiety of the amphetamine molecule resulted in a reduction in activity generally in accord with the above observations. Mescaline and fenfluramine depleted brain glycogen by less than 25% when administered in subconvulsant doses.

Somewhat unexpected results were obtained following the i.p. administration of the catecholamines. A highly significant increase in the concentration of brain glycogen occurred after the injection of noradrenaline or adrenaline and this increase was sustained for at least 60 min. 5-Hydroxytryptamine on the other hand did not alter the glycogen content. These results are not in accord with the observations reported in other investigations. The concentration of cerebral glycogen was unaffected by the administration of adrenaline to the dog (Kerr and Ghantus, 1936), rabbit (Kerr et al., 1937) and the mouse (Estler and Ammon, 1965), although Chance and Walker (1954) reported a transient increase in the glycogen content of the mouse brain after an i.v. injection of adrenaline.

In the doses used, adrenaline, noradrenaline and 5-hydroxytryptamine all produced an equivalent degree of sedation, albeit not a particularly severe sedation. As the concentration of glycogen was increased by noradrenaline and adrenaline but not by 5-hydroxytryptamine, the increase would appear to be due to an action mediated by the catecholamines rather than to the depression of activity.

Of the miscellaneous drugs, only relatively large doses of LSD and methysergide caused a decrease in the concentration of cerebral glycogen. Morphine, nicotine and phencyclidine did not deplete brain glycogen in subconvulsant doses.

The results of these experiments suggest primarily that for extensive cerebral glycogenolytic activity, a β-phenylethylamine nucleus is necessary. Maximum stimulation of glycogenolysis occurred with a cyclopropyl group on carbons 1 and 2 (tranylcypromine) or a methyl group on carbon 1 (amphetamine) of the alkylamine chain. Almost total loss of activity occurred if a substitution was made on the benzene ring or the isopropyl chain of amphetamine. Thus the integrity of these two structures appears to be important for cerebral glycogenolytic activity in the mouse.

ACKNOWLEDGEMENT

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