A Study of the Neurohumoral Control of Glycolysis in the Mouse Brain *in vivo*: Role of Noradrenaline and Dopamine

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Noradrenaline, Dopamine, Glycolysis, Adenyl Cyclase

Intraventricularly injected noradrenaline, dopamine and isoprenaline increased glycolysis as shown by the decrease in the concentration of "free" glycogen and increase in the concentration of lactate.

The effects of noradrenaline and isoprenaline were reduced in mice which had been pretreated with α-methyl-*p*-tyrosine. DL-Propranolol blocked the increase in glycolysis caused by noradrenaline, isoprenaline, sodium fluoride and analogues of 3,5-cyclic adenosine monophosphate.

It is suggested that the results of this investigation can be explained by the various drugs and neurohormones acting on the adenyl cyclase system *in vivo*, either by blocking the action of the neurohormone on the membrane bound enzyme or by antagonizing the effect of 3,5-cyclic adenosine monophosphate on glycolysis.

Introduction

It is generally assumed that noradrenaline activates membrane bound adenyl cyclase which brings about the intracellular synthesis of 3,5-cyclic AMP [1]. Cyclic AMP then produces an appropriate physiological response, the nature of which depends on the function of the tissue. The concentration of cyclic AMP is determined not only by its rate of synthesis but also by its rate of destruction by intracellular phosphodiesterases [2]. Therefore it is possible for drugs to influence brain glycolysis by affecting the availability of the neurohormone to adenyl cyclase, by directly affecting the activity of this enzyme or by increasing the rate of metabolism of cyclic AMP by phosphodiesterase. This study was therefore undertaken using various drugs and neurohormones to manipulate the activity of the enzyme system *in vivo* to see what effects such changes had on brain glycolysis.

In previous investigations into the mode of action of a number of β-adrenoreceptor blocking drugs on mouse brain glycolysis, no relationship could be found between their peripheral β-blocking properties and their effects on brain carbohydrate metabolism [3–6]. Indeed, propranolol appeared to be unique in its action on brain glycolysis. As part of the present study, it was therefore hoped to determine more precisely how propranolol acts.

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Methods

Specific pathogen free albino mice of the Alderley Park strain (18–22 g, either sex) were used throughout these experiments. The mice were injected with the drug or vehicle (control group) and the oesophageal temperatures were determined at regular intervals during the experimental period by means of a thermistor probe (Light and Sons, Brighton). If the temperature was reduced by more than 0.5 °C the animals were kept in a constant temperature room at 38 °C until they were killed. Hyperthermia only occurred to a slight extent after the administration of caffeine.

At various times (shown in Results) after administration of the drug, groups of at least 5 mice were killed by immersion in liquid nitrogen. After thorough freezing, the mice were decapitated, their brains were rapidly chipped out, weighed and triturated with a protein precipitating agent (generally 10% trichloracetic acid) in a cooled glass mortar. After centrifugation at approximately $500 \times g$ for 10–15 min the supernatant fraction was separated from the pellet; both fractions were kept at 0 °C until the assays were undertaken. With the exception of glycogen, the assays were performed on the same day as the extracts were prepared. The following determinations were made.

Glycogen. The trichloracetic acid soluble ("free") and insoluble ("bound") glycogen was treated by the method described by Russel and Bloom [7] and the glucose formed after acid hydrolysis estimated by the glucose oxidase method of Hugget and Nixon [8].

Glucose and lactate were estimated by the glucose oxidase method [8] and lactic dehydrogenase methods [9] respectively, on aliquots of the supernatant fraction.

Statistical analysis of results. Results were calculated as μmol/g weight of brain. However, to compare the effects of several different drugs on the same biochemical parameter, the results are expressed as percentage change relative to the control. The statistical significance was assessed using Students t-test.

Results

Effect of sodium fluoride and some synthetic analogues of 3,5-cyclic adenosine monophosphate on brain glycolysis

Sodium fluoride has been shown to stimulate the adenyl cyclase system of the rat pineal gland [10]. This halide was therefore injected intraventricularly (50 μg in 10 μl) into groups of mice. Within 1 – 2 sec of injection it caused hyperexcitability and on some cases clonic-tinic convulsions which lasted for 10 – 20 sec. All the animals recovered but were behaviourally depressed (decreased exploration exploration of cages remaining in corner of cage unless disturbed, slowed righting reflex) for the duration of the experiment. Lower doses of fluoride (10 and 25 μg) had qualitatively similar effects.

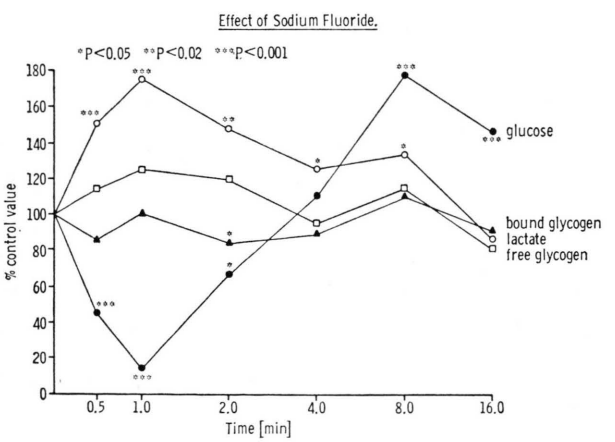

Fig. 1. Effect of sodium fluoride on mouse brain glycolysis. Sodium fluoride injected into the lateral ventricles of conscious mice (50 μg in 10 μl). Results expressed as a percentage of the control value. Each point represents the mean of at least 5 animals. Significance of the difference from control values given by $*P<0.05$; $**P<0.025$; $***P<0.01$. Control values ($\pm$s.e.m.) for "free" glycogen, 0.720 ± 0.025 μmol/g (as glucose); "bound" glycogen, 1.375 ± 0.012 μmol/g (as glucose); glucose, 0.530 ± 0.040 μmol/; lactate, 2.272 ± 0.070 μmol/g.

It is apparent from the results that sodium fluoride increases the concentration of brain lactate and reduces that of glucose affecting the concentration of glycogen (Fig. 1). The peak effect occurred 1 min after injection when the behavioural symptoms were most pronounced.

The effects of sodium fluoride were also studied in groups of animals which had been pretreated for up to 4 hours with DL-propranolol (10 mg/kg i.p.). The halide was then injected intraventricularly (50 μg) one min before the mice were killed. Propranolol did not appreciably affect the symptoms produced by sodium fluoride but it did antagonize most of the effects of the halide on brain lactate and glucose (Fig. 2).

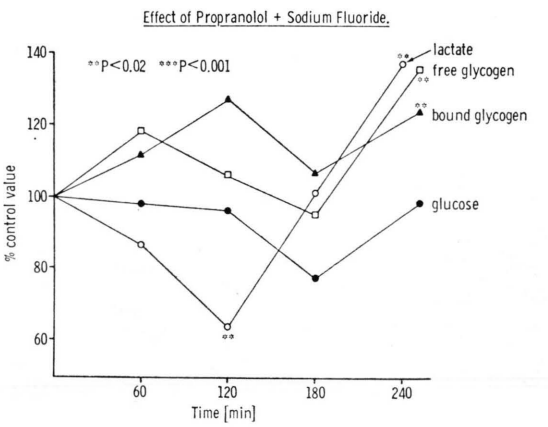

Fig. 2. Effect of propranolol and sodium fluoride on mouse brain glycolysis. Group of mice pretreated for 60, 120, 180 and 240 min with DL-propranolol (10 mg/kg i.p.). Two min before being killed, sodium fluoride was injected into the lateral ventricles (50 μg in 10 μl). Details otherwise as shown in Fig. 1.

3,5-cyclic AMP *, dibutyryl 3,5-cyclic AMP, monobutyryl 3,5-cyclic AMP and 3,5-cyclic IMP were also investigated for their effects on brain glycolysis. All were injected intraventricularly in a dose of 10 μg/10 μl. The behavioural effects were similar; the animals were depressed for up to 8 min after injection. However, those mice injected with 3,4-cyclic IMP were hyperactive for up to two min following injection; this was succeeded by behavioural depression.

The effects of these nucleotides on brain glycolysis were qualitatively similar. All the substances increased the concentration of glycogen and lactate

* *Abbreviations:* 3,5-cyclic AMP = 3′,5′-cyclic adenosine monophosphate; 3,5-cyclic IMP = 3′,5′-cyclic inosine monophosphate.

and decreased that of glucose for up to 3 min following injection. These changes were followed by a reduction in lactate and increase in glucose. The effects of 3,5-cyclic AMP are shown in Fig. 3.

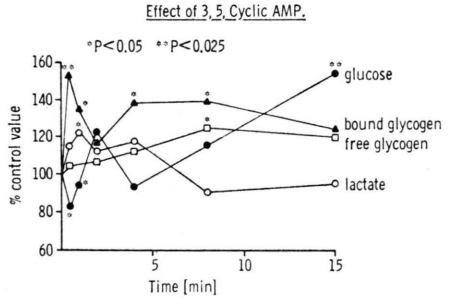

Fig. 3. Effect of 3,5-cyclic AMP on mouse brain glycolysis. Mice injected into the lateral ventricles with cyclic AMP (10 μg in 10 μl). Details otherwise as shown in Fig. 1.

Quantitatively, the most marked changes in carbohydrate metabolism were seen after the intraventricular injection of dibutyryl 3,4-cyclic AMP (Fig. 4).

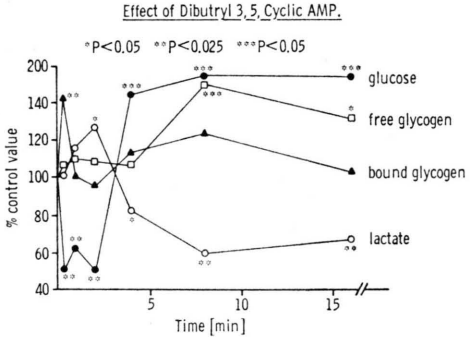

Fig. 4. Effect of dibutyryl-3,5-cyclic AMP on mouse brain glycolysis. Mice injected into the lateral ventricles with dibutyryl-3,5-cyclic AMP (10 μg in 10 μl). Details otherwise as shown in Fig. 1.

The effect of DL-propranolol on the changes induced by dibutyryl-3,5-cyclic AMP was also studied. In this experiment, groups of mice were treated for up to 4 hours with propranolol. Two min before being killed, and at 60, 120, 180 and 240 min after treatment with propranolol, the mice were injected intraventricularly with dibutyryl 3,5-cyclic AMP. Propranolol did not appreciably reduce the behavioural changes produced by the cyclic AMP derivative but it did significantly antagonize most of the effects this nucleotide on brain carbohydrate metabolism (Fig. 5).

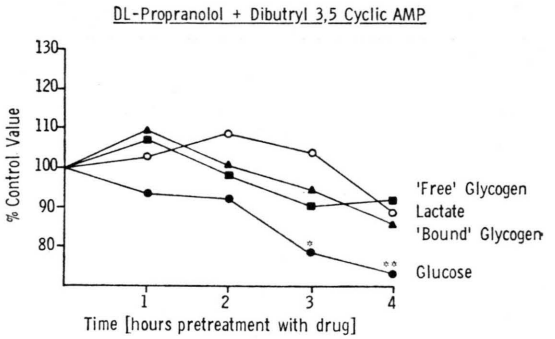

Fig. 5. Effect of DL-propranolol and dibutyryl-3,5-cyclic AMP on mouse brain glycolysis. Groups of mice pretreated for 60, 120, 180 and 240 min with DL-propranolol (10 mg/kg i.p.). Two min before being killed the mice were injected into the lateral ventricle with dibutyryl-3,5-cyclic AMP (10 μg in 10 μl). Details otherwise as shown in Fig. 1.

The effect of propranolol on the changes in brain glycolysis induced by noradrenaline, isoprenaline and α-methyl p-tyrosine

Previous studies (Leonard, unpublished) have shown that the maximal increase in brain glycolysis occurred 1 and 2 min respectively after the intraventricular administration of 5 μg of noradrenaline or isoprenaline. In this and in all subsequent experiments, intraventricular injections into conscious mice were made using the method of Haley and McCormick [11]. Groups of mice were pretreated with DL-propranolol alone (20 mg/kg i.p.) or in combination with α-methyl-*p*-tyrosine (α-MPT; 240 mg/kg in 4 equally divided doses over a period of 24 hours).

It is clear from the results that when the synthesis of noradrenaline is blocked by pretreatment of the mice with α-MPT, glycolysis is considerably reduced (Table I). This effect of α-MPT is antagonized to some extent by the intraventricular administration of noradrenaline and isoprenaline. Propranolol reduced brain lactate and increased glucose and glycogen; it antagonized the effects of both noradrenaline and isoprenaline. It did not appreciably affect the reduction in glycolysis produced by α-MPT.

These results suggest that brain glycolysis might be controlled by an adrenergic mechanism. To investigate the mechanisms underlying glycolysis further it was necessary to study the effects of the precursors of noradrenaline.

Table I. Changes in brain glycolysis produced by noradrenaline, isoprenaline, α-methyl-*p*-tyrosine and propranolol.

No.	Group	Treatment time	Glycogen "Free"	Glycogen "Bound"	Glucose	Lactate
1.	Controls (saline injected into ventricles	—	0.711 ± 0.025	1.36 ± 0.12	0.573 ± 0.03	2.38 ± 0.095
2.	Noradrenaline (5 μg injected into ventricles	1 min	0.569 ± 0.029 **	0.929 ± 0.098	0.503 ± 0.09	3.34 ± 0.132 *
3.	Isoprenaline (5 μg injected into ventricles	2 min	0.461 ± 0.031 **	0.898 ± 0.036 **	0.532 ± 0.018	3.26 ± 0.109 *
4.	DL-propranolol (20 mg/kg i.p.)	120 min	0.911 ± 0.067 **	2.25 ± 0.32 ***	1.125 ± 0.16 ***	1.35 ± 0.121 ***
5.	Noradrenaline + DL-propranolol	1 min + 120 min	1.034 ± 0.099 ***	1.846 ± 0.14 **	0.973 ± 0.077 **	1.93 ± 0.09 *
6.	Isoprenaline + DL-propranolol	2 min + 120 min	0.96 ± 0.059 **	0.970 ± 0.10 *	0.575 ± 0.048	2.02 ± 0.10
7.	α-methyl-*p*-tyrosine (240 mg/kg)	24 h	0.956 ± 0.065 **	2.352 ± 0.216 ***	1.176 ± 0.108 ***	1.81 ± 0.05 **
8.	α-methyl-*p*-tyrosine + DL-propranolol	24 h + 120 min	0.986 ± 0.033 ***	2.30 ± 0.096 ***	1.150 ± 0.048 ***	0.92 ± 0.73 ***
9.	α-methyl-*p*-tyrosine + noradrenaline	24 h + 1 min	0.83 ± 0.042 *	2.422 ± 0.136 ***	0.634 ± 0.068 *	1.86 ± 0.230 **
10.	α-methyl-*p*-tyrosine + isoprenaline	24 h + 2 min	0.76 ± 0.065	1.738 ± 0.09 *	0.526 ± 0.023	2.29 ± 0.173 *

Each result represents the mean ± s.e.m. of at least 5 mice per group. The significance of the results shown by $*P<0.05$; $**P<0.01$; $***P<0.001$. Noradrenaline and isoprenaline were injected into the lateral ventricles in physiological saline. Total volume injected was 10 μl.

The effect of dihydroxyphenylalamine (dopa), dopamine, diethyldithiocarbamate and apomorphine on brain glycolysis

Dopa, when administered intraperitoneally to mice at a dose of 300 mg/kg, caused salivation, sweating and slight hyperexcitability in some of the animals. However, 60 min after injection these effects had been succeeded by marked behavioural depression and hypothermia. Lower doses (200, 100 and 50 mg/kg) caused only slight symptoms although hypothermia occurred following the administration of 200 mg/kg. The changes shown in Fig. 6 were not, however, a consequence of hypothermia as these animals were kept at an ambient temperature of 38 °C for the duration of the experiment.

From these results it is apparent that dopa increases glycolysis for at least 120 min after its administration (Fig. 6). This is shown by the rise in lactate and fall in glucose and "bound" glycogen. There appears to be a compensatory increase in glycogenesis 120 min after the administration of the drug.

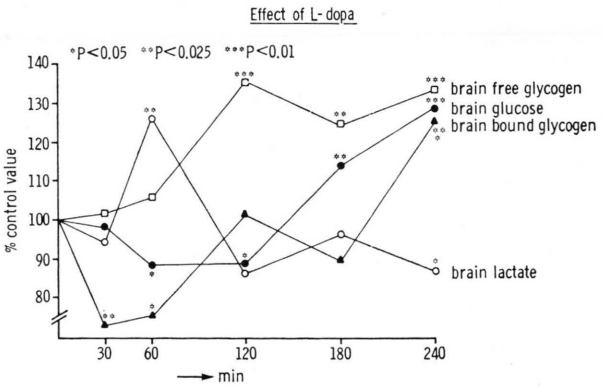

Fig. 6. Effect of Dopa on mouse brain glycolysis. Dopa given in a dose of 300 mg/kg i.p.

Dopamine was given into the lateral ventricles (10 μg in 10 μl of physiological saline) and the animals killed at different times for up to 30 min after injection. The same volume of physiological saline caused only slight behavioural depression which lasted for approximately 2 min. One min after dopamine however, the animals were depressed and showed occasional ear twitching. Two min later the animals were more severly depressed but hyper-

sensitive to touch when disturbed. They gradually recovered during the remainder of the experimental period.

The changes in brain carbohydrate metabolism were most marked during the first 8 min following the injection of dopamine (Fig. 7), this coincided with the period during which the behavioural changes were most marked. There was a marked decrease in the concentration of brain glucose and glycogen and a slight rise in the concentration of lactate.

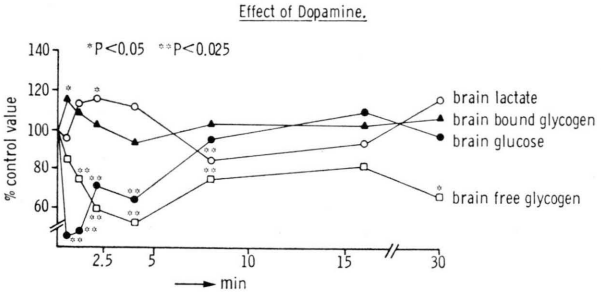

Fig. 7. Effect of dopamine on mouse brain glycolysis. Dopamine injected into lateral ventricles (10 μg in 10 μl). Details otherwise as given in Fig. 1.

The action of diethyldithiocarbamate (400 mg/kg i.p.) was investigated, as this drug blocks dopamine β-oxidase activity thereby raising endogenous brain dopamine and reducing noradrenaline levels [12]. Mice injected with this drug and kept at room temperature were behaviourally depressed and hypothermic. However, at an ambient temperature of 38 °C the animals were not depressed.

The results, summarized in Fig. 8 (which were obtained from mice kept at 38 °C) show that diethyldithiocarbamate had an effect which was quite unlike dopamine and dopa. This is indicated by the rise in brain glucose and "bound" glycogen and fall in lactate. It is however, difficult to explain the marked decrease in "free" glycogen which occurs for the first 120 min after the drug was administered. As diethyldithiocarbamate is known to inhibit other enzyme systems [13, 14] it is difficult to draw any firm conclusions from the present findings as to its action on brain glycolysis.

There is considerable evidence to suggest that apomorphine acts directly on dopamine receptors in the central nervous system without appreciably affecting the endogenous concentrations of the amine [15, 16]. This drug was therefore investigated for its effect on brain glycolysis.

The administration of apomorphine (25 mg/kg i.p.) caused the mice to become very hyperexcitable, move rapidly around the cage and periodically to show stereotyped circling activity. Slight ptosis was apparent 30 min after injection. These symptoms were less marked 60 min after injection, and after 120 min most activity appeared to be confined to stereotyped "grooming". The mice were then behaviourally rather depressed for the remainder of the experimental period. No change in the oesophageal temperature was noted during the experiment. Lower doses of apomorphine (5 and 10 mg/kg) caused some hyperactivity and stereotyped behaviour but the effects were less marked.

The results of the changes in brain carbohydrate metabolism seen after apomorphine (25 mg/kg) show that this drug stimulates glycolysis as indicated by the elevated lactate and reduced glucose levels (Fig. 9). Glycogen levels did not alter significantly during the experiment.

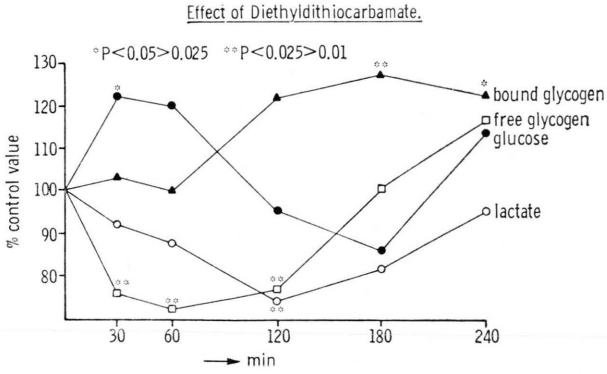

Fig. 8. Effect of diethyldithiocarbamate on mouse brain glycolysis. Diethyldithiocarbonate given in a dose of 400 mg/kg (i.p.). Details otherwise as given in Fig. 1.

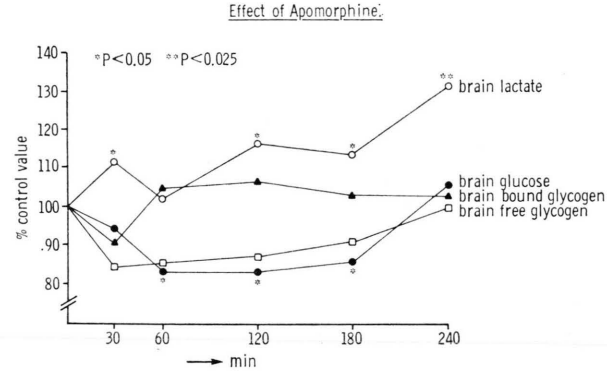

Fig. 9. Effect of apomorphine on mouse brain glycolysis. Apomorphine given in a dose of 25 mg/kg (i.p.). Details otherwise as given in Fig. 1.

Discussion

The results of this investigation support the studies of others [17, 18] that noradrenaline plays an important role in activating the adenyl cyclase system in central nervous tissue. In addition to finding that both noradrenaline and isoprenaline increase glycolysis when injected into the cerebral ventricles, the β-adrenoceptor blocking drug DL-propranolol was found to decrease this effect of these amines. Propranolol has been shown to inhibit the activation of rat pineal gland adenyl cyclase activity by noradrenaline *in vitro* [10]. There is also some evidence to show that DL-propranolol reduces the *in vivo* concentration of brain 3,5-cyclic AMP in the grouped mice (Somerville, unpublished). The precise site of action of propranolol on the adenylcyclase system is by no means certain. However, from *in vitro* studies D-propranolol, which is almost devoid of β-adrenoreceptor blocking activity, also inhibits cyclase activity (Somerville, unpublished). D-Propranolol has also been shown to reduce brain glycolysis *in vivo* (Leonard, unpublished) and to be approximately equi-active with DL-propranolol as a local anaesthetic agent [19]. Thus it seems possible that propranolol reduces brain glycolysis by blocking the action of noradrenaline on central adrenergic receptors, thereby reducing cyclase activity, and also by stabilising the neuronal membrane, thereby reducing nervous activity. It is also possible that the effects on the concentration of brain glucose, lactate and glycogen which were observed after the administration of propranolol and α-methyl-*p*-tyrosine could have been influenced by changes in the uptake of glucose into the brain. Clearly this possibility will have to be studied further before more firm conclusions can be reached concerning the mechanism of action of propranolol on brain carbohydrate metabolism.

It was surprising to find that propranolol also antagonized the actions of intraventricularly administered dibutyryl-3,5-cyclic AMP, a substance which mimics the action of 3,5-cyclic AMP as the "second" messenger in most tissues [20]. Such an effect of propranolol might suggest that it has two sites of action in the control of brain glycolysis. One is extracellular, blocking the action of the neurohormone, and the second is intracellular in inhibiting the action of cyclic AMP. It is possible, however, that the relative specificity of propranolol in blocking the neurohormone differs from that required to block the action of cyclic AMP. Other investigations have shown that propranolol and other β-blockers inhibit the increase in lipolysis elicited by dibutyryl-3,5-cyclic AMP only in concentrations which probably inhibit the general metabolism of the adipose tissue [21]. It is clear from ionophoretic studies that the excitation of neurones in the cerebral cortex of the cat by noradrenaline is easily and selectively antagonized by such β-adrenoceptor blockers as propranolol, sotalol and INPEA [22]. Such findings may be additional evidence for the view that the primary action of propranolol is on adenyl cyclase in the nerve cell membrane; secondary effects on the intracellular metabolism might arise when relatively high doses of the drug are given.

Besides its ability to antagonise the actions of dibutyryl-3,5-cyclic AMP, propranolol also prevents the increased glycolysis which results from the intraventricular administration of sodium fluoride. In this respect, the action of the drug differs markedly from its effect on the response of pineal gland adenyl cyclase to fluoride ions. Thus, Weiss [10] found that propranolol had no effect while the β-adrenergic blocking drug dichloisoprenaline actually potentiated the stimulation of adenyl cyclase activity caused by sodium fluoride. Weiss [10] therefore suggested that, in the pineal gland, the cyclase systems responding to catecholamines and to sodium fluoride have may characteristics in common but that the specific site at which these compounds interact to produce their effects are different. Such a conclusion cannot be so readily drawn from the present results where propranolol is almost equally as effective in antagonizing the halide and dibutyryl-cyclic AMP induced changes in glycolysis.

It is evident from the results of other investigators that drugs which are known to specifically block α- or β-adrenoceptors in peripheral tissues have qualitatively similar effects in their actions on the brain, for example in blocking the excitatory action of noradrenaline on single neurones [22], or in blocking the activity of adenyl cyclase in the rat pineal gland [24]. Recent studies have also shown that propranolol has a similar effect to phentolamine and phenoxybenzamine on some parameters of glycolysis in the mouse brain [23]. As both noradrenaline and isoprenaline are equally effective in increasing brain glycolysis, an effect which is antagonised by propranolol, it seems unlikely that the clear concepts of α- and β-receptors as applied to other tissues can be applied to the brain.

Besides noradrenaline, dopamine may also have a role to play in the control of brain glycolysis. This is suggested by the finding that both dopa and dopamine increase glycolysis and that qualitatively similar effects were found following the parenteral administration of apomorphine. Apomorphine has been shown by others to directly stimulate dopamine receptors [15, 16]. As it is well established that the regional distribution of dopamine and noradrenaline differ, it is possible that their effect on brain adenyl cyclase activity has some physiological relevance. In this respect, it is of interest that others have found little correlation between the regional distribution of noradrenaline, histamine and adenyl cyclase in brain [25].

Although the present results were obtained *in vivo*, criticism can be justifiably made of the unphysiological conditions under which the neurohormones were administered and also the relatively high doses of some of the drugs used to produce the observed changes. Nevertheless, such a study may provide a useful starting point for further work which could lead to a better understanding of the mechanisms by which centrally acting drugs can affect brain carbohydrate metabolism.

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