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A COMPARISON BETWEEN THE EFFECTS OF PHENOXYBENZAMINE, PHENTOLAMINE AND PROPRANOLOL ON MOUSE BRAIN GLYCOLYSIS

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Abstract—Propranolol reduced brain lactate and pyruvate concentrations. Phentolamine raised brain pyruvate whereas phenoxybenzamine was without effect. Phentolamine produced a biphasic change in brain lactate whereas phenoxybenzamine did not affect the concentration of this metabolite. Propranolol reduced glucose-6-phosphate but did not affect the concentration of fructose-1,6-diphosphate. Both of the α-adrenergic blocking drugs raised the brain concentrations of these metabolites. Propranolol increased the concentration of "bound" glycogen without affecting that of the "free" form. In contrast, phentolamine produced a biphasic change in "free" glycogen and slightly reduced the "bound" form. Phenoxybenzamine reduced the concentration of "free" glycogen without affecting the "bound" form.

THERE is evidence from studies *in vivo* that the actions of noradrenaline on the brain can be antagonized by both α- and β-adrenaline receptor blocking drugs.[1–3] To investigate this problem further, and as part of a general investigation of the effects of adrenoceptive blocking drugs on carbohydrate metabolism in the mouse brain, the effects of *dl*-propranolol were compared with those of phentolamine and phenoxybenzamine. A comparison between these drugs seemed appropriate as it is well established that they readily enter the brain[4–9] and are also potent adrenaline receptor blocking drugs in most peripheral tissues.

MATERIAL AND 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 determined at regular intervals during the experimental period by means of a thermistor probe (Light & Sons, Brighton). If the temperature of the mice in any of the groups was reduced by more than 0·5°, the animals were placed in a constant temperature room at 38° until they were killed. Hyperthermia did not occur with any of the drugs tested.

At various times (shown in Results) after administration of the drug, groups of at least five mice were killed by immersion in liquid nitrogen. The mice were decapitated, their brains rapidly chipped out while still frozen, weighed and triturated with a protein precipitating agent (generally 10% (w/v) trichloracetic acid) in a cooled glass mortar. After centrifugation (approximately 500 *g* for 10–15 min) the supernatant fraction was separated from the pellet. Both fractions were kept on an ice bath until

the assays were undertaken. With the exception of glycogen, the assays were performed on the same day that the extracts were prepared. The following determinations were made:

Glycogen. The trichloracetic acid soluble glycogen ("free") and insoluble glycogen ("bound") were treated by the method described by Russell and Bloom.[10] After hydrolysis of the glycogen with N sulphuric acid, and neutralization of the extract, the glucose formed was estimated by the glucose oxidase method of Hugget and Nixon.[11]

Brain glucose. The protein precipitant described by Gey[12] was used and the glucose content of the supernatant solution determined by the glucose oxidase method.

Brain lactate and pyruvate were estimated on portions of the supernatant fraction following protein precipitation (with 10% trichloracetic acid for lactate and 0·6 N perchloric acid for pyruvate) by the lactate dehydrogenase method of Scholz *et al.*, using Boehringer test kits.

Brain glucose-6-phosphate and fructose-1,6-diphosphate were determined on portions of the supernatant solution, following protein precipitation (with 10% trichloracetic acid), by the spectrophotometric method of Hohorst[14] for glucose-6-phosphate and of Slater[15] for fructose-1,6-diphosphate.

Brain hexokinase activity. Following decapitation of the mice, total brain hexokinase activity was determined in fresh half brains by the method of Slein *et al.*[16] as modified by Bennet *et al.*[17]

The significance of the results was assessed using Student's *t*-test.

RESULTS

None of the drugs tested had any marked effect on the gross behaviour of the mice.

The results are shown for one dose level only of the three drugs. Lower doses (10 and 20 mg/kg) of the α-blockers produced qualitatively similar but less marked effects on mouse brain glycolysis. Propranolol (3 mg/kg) caused a slight reduction in glycolysis but no significant change in glycogenesis.

Effect on brain glycogen. Propranolol had no significant effect on "free" glycogen but increased the concentration of "bound" glycogen 120 min after administration (Table 1). Phenoxybenzamine caused a transitory decrease in "free" glycogen without significantly affecting "bound" glycogen; phentolamine caused a biphasic change in "free" glycogen and a transitory decrease in "bound" glycogen.

*Effect on brain glucose, glucose-6-phosphate and fructose-1,6-diphosphate. dl-*Propranolol caused a significant increase in the concentration of glucose which persisted for 180 min following the administration of the drug (Table 1). This drug had a clear effect on glucose-6-phosphate levels but did not significantly change fructose diphosphate levels.

Phentolamine caused a marked rise in brain glucose, glucose-6-phosphate and fructose diphosphate levels. Following the administration of phenoxybenzamine, brain glucose was reduced and glucose-6-phosphate raised; the concentration of fructose diphosphate levels were raised 120 min after the drug had been administered and then rapidly returned to the control levels.

Effect on brain pyruvate and lactate. Propranolol caused a prolonged decrease in the concentration of brain lactate and a slight decrease in pyruvate (Table 1). Phentolamine significantly increased brain pyruvate and produced a biphasic change in brain lactate, the initial slight fall being followed by a compensatory rise in lactate 120 min

TABLE 1. EFFECT OF *dl*-PROPRANOLOL, PHENOXYBENZAMINE AND PHENTOLAMINE ON MOUSE BRAIN GLYCOLYSIS

Drug	Time after drug administration (min)					
	0	30	60	120	180	240
dl-Propranolol (10)						
"Free" glycogen	0·70 ± 0·075	0·73 ± 0·06	0·77 ± 0·09	0·79 ± 0·045	0·74 ± 0·05	0·77 ± 0·085
"Bound" glycogen	1·70 ± 0·109	1·77 ± 0·096	1·87 ± 0·110	2·22† ± 0·090	2·24† ± 0·086	2·13* ± 0·125
Glucose	0·378 ± 0·061	0·470† ± 0·05	0·45* ± 0·07	0·485† ± 0·065	0·490† ± 0·04	0·405 ± 0·07
Glucose-6-phosphate	0·082 ± 0·01	0·052† ± 0·008	0·051† ± 0·01	0·052† ± 0·009	0·066* ± 0·016	0·072 ± 0·02
Fructose diphosphate	0·0304 ± 0·003	0·0303 ± 0·002	0·030 ± 0·002	0·0274 ± 0·002	0·272 ± 0·001	0·0268 ± 0·001
Pyruvate	0·111 ± 0·02	0·113 ± 0·03	0·104 ± 0·04	0·077† ± 0·03	0·095 ± 0·05	0·100 ± 0·055
Lactate	2·235 ± 0·19	1·662† ± 0·15	1·230† ± 0·16	1·235‡ ± 0·20	1·382‡ ± 0·18	1·790* ± 0·24
Phentolamine (30)						
"Free" glycogen		0·86* ± 0·054	1·05† ± 0·075	0·56* ± 0·068	0·72 ± 0·06	0·71 ± 0·07
"Bound" glycogen		1·45* ± 0·080	1·38* ± 0·060	1·54 ± 0·10	1·48 ± 0·09	1·62 ± 0·10
Glucose		0·454 ± 0·06	0·605‡ ± 0·04	0·358 ± 0·05	0·394 ± 0·07	0·380 ± 0·05
Glucose-6-phosphate		0·084 ± 0·02	1·230‡ ± 0·03	1·115‡ ± 0·025	0·0965 ± 0·02	0·0835 ± 0·025
Fructose diphosphate		0·0396‡ ± 0·004	0·0383‡ ± 0·003	0·0377‡ ± 0·002	0·0398‡ ± 0·005	0·0305 ± 0·003
Pyruvate		0·113 ± 0·03	0·142* ± 0·025	0·122 ± 0·03	0·135* ± 0·02	0·130 ± 0·040
Lactate		2·180 ± 0·16	1·987 ± 0·20	1·760* ± 0·150	2·43 ± 0·23	2·70* ± 0·180
Phenoxybenzamine (30)						
"Free" glycogen		0·675 ± 0·06	0·734 ± 0·09	0·492† ± 0·10	0·684 ± 0·08	0·69 ± 0·072
"Bound" glycogen		1·66 ± 0·078	1·57 ± 0·098	1·69 ± 0·102	1·73 ± 0·087	1·85 ± 0·089
Glucose		0·300† ± 0·050	0·314 ± 0·090	0·292† ± 0·04	0·320 ± 0·086	0·350 ± 0·077
Glucose-6-phosphate		0·086 ± 0·008	0·091 ± 0·009	0·114‡ ± 0·006	0·123‡ ± 0·007	0·117‡ ± 0·005
Fructose diphosphate		0·029 ± 0·002	0·032 ± 0·004	0·034* ± 0·001	0·0318 ± 0·003	0·0309 ± 0·004
Pyruvate		0·109 ± 0·02	0·114 ± 0·03	0·108 ± 0·02	0·117 ± 0·04	0·107 ± 0·06
Lactate		2·228 ± 0·210	2·204 ± 0·104	2·231 ± 0·100	2·218 ± 0·103	2·238 ± 0·091

Each result represents the mean ± S.E.M. of at least 5 mice. The significance of the difference between the drug treated and the control group shown by * $P < 0·05$; † $P < 0·01$; ‡ $P < 0·001$. All results as μmoles/g wet weight brain. Values shown in parentheses give dose, in mg/kg, of drug injected. Control group (0) injected with physiological saline. All values for drug treated groups compared with control (0 min) group.

later. In contrast, phenoxybenzamine did not significantly affect the concentration of either brain pyruvate or lactate.

Effect on brain hexokinase. Neither phenoxybenzamine nor propranolol had any effect on total brain hexokinase activity. However, phentolamine produced a slight (12 per cent) decrease in hexokinase activity 4 hr after the drug had been administered. From the present investigation, the significance of this finding is difficult to assess.

These results therefore suggest that whereas propranolol decreases glycolysis; both α-blockers increase it.

DISCUSSION

The results of this investigation show that a distinction can be made between the effects of the two α-adrenaline receptor blocking drugs and propranolol. However, it is apparent from the previous study[4] that the changes in several of the parameters produced by the α-adrenergic blocking drugs are qualitatively similar to those found for other β-blocking drugs. For example, phenoxybenzamine produces changes in glycogen, glucose, pyruvate and lactate similar to those found following the administration of sotalol. As both these drugs increase glycogenolysis and glycolysis, it is clear that they have effects on brain carbohydrate metabolism similar to central stimulant drugs. However, there is little evidence from their effects on gross behaviour of mice that they cause hyperactivity. Indeed, sotalol has been found to increase the hexobarbitone induced sleeping time of mice, which suggests that it has a sedative rather than a stimulant effect.[18]

The mechanisms by which adrenaline receptor blocking drugs affect glycolysis may involve their effect on the concentration, or turnover, of brain catecholamines. It is well established that the control of glycolysis in the brain and other tissues is mediated by the adenyl cyclase system.[19] Clearly, any drug affecting the turnover of noradrenaline, which acts as the principal neurohormone in activating the membrane bound enzyme,[20] will affect the rate of brain glycolysis. There is evidence that when propranolol is administered daily for 4 days to rodents, it does produce a slight reduction in the concentration of brain noradrenaline,[21] although no evidence could be found that its administration immediately affects the turnover of this amine.[4] Nevertheless, there is evidence that propranolol reduces the concentration of 3,5 cyclic adenosine monophosphate *in vivo*,[22] presumably by blocking the action of the neurohormone on adenyl cyclase. This finding could account for the glycogenesis and reduced glycolysis found in mice following their treatment with this drug. Conversely, the "stimulant" profile produced by phenoxybenzamine could be related to its ability to increase the synthesis of noradrenaline, possibly by increasing tyrosine hydroxylase activity.[9] Furthermore, Bigelow and coworkers[8] have shown that the turnover of catecholamines is increased in rats following the administration of phenoxybenzamine even though the animals were behaviourally depressed. Such results might explain the increase in glycolysis found in the present investigation following the administration of this drug to mice.

So far there is little to suggest that phentolamine affects the concentration or turnover of brain catecholamines *in vivo*. However, Weiss[3] has shown that this drug reduces adenyl cyclase activity in the rat pineal gland *in vitro*. Phenoxybenzamine had a similar effect but neither of these drugs was as effective as propranolol in antagonizing the noradrenaline induced increase in cyclase activity in this tissue.

The results of this study show that it is possible to distinguish α- from β-adrenaline receptor blocking drugs by their effects on mouse brain glycolysis. However, the diverse effects on carbohydrate metabolism shown in earlier studies[4–6] suggest that these criteria alone do not provide an unequivocal distinction between α- and β-blockers. It is possible that the sites of action of these drugs in the brain differ so that any fundamental differences may only be detectable in discrete areas. Future work will, therefore, be directed towards comparing the effects of different adrenergic agonists and antagonists on anatomically discrete areas of the mouse brain in the hope that such an approach may provide a fuller understanding of the nature of adrenergic receptor(s) in the central nervous system.

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