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THE EFFECT OF PHYSOSTIGMINE AND NEOSTIGMINE ON THE CONCENTRATION OF GLYCOGEN IN DIAPHRAGM AND TRICEPS MUSCLE OF THE RAT

B. B. MRŠULJA, M. TERZIĆ and V. M. VARAGIĆ

Department of Pharmacology, and Department of Biochemistry,
Medical Faculty, Belgrade 7, Yugoslavia

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Abstract—Physostigmine (100–400 μg/kg) was found to produce a dose-dependent decrease in the amount of glycogen in the diaphragm of the rat. In triceps muscle only a very high dose of physostigmine produced a decrease in the amount of glycogen. Neostigmine (50 μg/kg) did not affect glycogen in either of these muscles. Atropine (0·5 mg/kg) did not influence the glycogenolytic effect of physostigmine in the diaphragm, while propranolol (10 mg/kg) was able to block it. *In vitro*, the concentrations of physostigmine, which roughly corresponded to those used *in vivo*, did not significantly affect the amount of glycogen in the diaphragm. Very high concentrations of physostigmine produced a significant decrease in the amount of glycogen in the diaphragm *in vitro*. It is concluded that physostigmine produced glycogenolysis in the diaphragm through an activation of adrenergic mechanisms, most probably by a central action. Peripheral cholinergic processes, implicating muscarinic receptors, do not take part in the glycogenolysis in diaphragm.

THE ABILITY of physostigmine to produce a decrease in the concentration of glycogen in liver and in the various structures of the central nervous system of the rat has been already discovered.[1, 2] In contrast to physostigmine, neostigmine did not produce glycogenolysis in either the liver or the central nervous system. These effects were explained in terms of activation by physostigmine of the corresponding adrenergic mechanisms which are known to participate in glycogenolysis.

The contractions of the skeletal muscle, as for example in shivering, respond to two signals: a cholinergic one, triggering contraction of the muscle and an adrenergic one, probably supplying fuel for muscle work. On the other hand, the role of the sympathetic nervous system in the regulation of glycogenolysis in skeletal muscle is still debated.[3] Brodie *et al.*[4] have commented on the problem whether the sympathetic activity is needed to provide metabolic fuel for the normal activity of skeletal muscle. It has been found that animals the adrenergic nerve function of which has been blocked are not able to mobilize additional energy substrates and, for example can swim only half the time of the control animals.[5] When these animals are forced to exercise on a treadmill, they soon collapse from exhaustion.[6] The animals without adrenergic function are unable to utilize the skeletal muscle glycogen or increase plasma glucose and free fatty acid levels.[4]

It was therefore of interest to investigate the effect of two anticholinesterase agents, physostigmine and neostigmine, on the concentration of glycogen in two functionally different striated muscles such as the diaphragm and the triceps.

MATERIALS AND METHODS

Male and female albino rats (220–260 g) were used in these experiments. Standard food and water were available to all animals *ad lib.*

The animals were sacrificed by means of a specially constructed guillotine. For determination of glycogen in the diaphragm a piece of muscle of about 30 mg was cut out, taking care that the sample is cut always from the same anatomical region. The samples were taken within 60 sec after death of the animal. Under the same conditions a piece of triceps muscle, weighing about 40 mg, was also cut out and used for glycogen estimation.

In all the experiments physostigmine and neostigmine were injected intravenously. These anticholinesterases were allowed to act for 30 min before the animals were sacrificed. The control animals were treated with saline solution intravenously. Propranolol (10 mg/kg) and atropine (0·5 mg/kg) were injected intraperitoneally. These substances were allowed to act for 20 min before physostigmine or neostigmine were injected.

Glycogen was estimated according to the method described by Montgomery.[7]

The following substances were used: physostigmine salicylate, neostigmine methylsulphate, propranolol (Inderal*) and atropine sulphate.

RESULTS

The effect of physostigmine. Increasing doses of physostigmine were found to affect the amount of glycogen in the diaphragm and triceps muscle. All the investigated

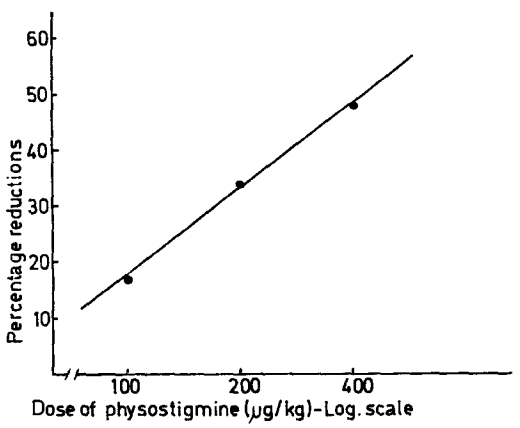

FIG. 1. Dose–response relationship between log doses of physostigmine and the percentage reduction in the amount of glycogen in diaphragm.

doses were found to produce a significant decrease in the glycogen concentration in the diaphragm ($P < 0{\cdot}001$). This decrease was found to be dose-dependent. A straight line was obtained if the percentage reductions in the amount of glycogen in the diaphragm were plotted against log doses (Fig. 1).

On the other hand, small and medium doses of physostigmine produced an insignificant fall of the amount of glycogen in triceps muscle. However, a large dose of

* Kindly supplied by Dr A. Spinks from I.C.I., Macclesfield, England.

physostigmine (400 μg/kg) was able to produce a significant decrease in the amount of glycogen in this muscle (Table 1).

The effect of neostigmine. Neostigmine was injected either in doses of 50 or 100 μg/kg. The dose of 50 μg/kg did not produce any change in the amount of glycogen in both diaphragm and triceps muscle. Thus, in a group of four animals, treated by this dose of neostigmine, 358·5 ± 9·7 mg% of glycogen was found in diaphragm and 524·7 ± 10·3 mg% in triceps muscle. These values are very close to those found in control animals.

The dose of 100 μg/kg neostigmine was found to be lethal for about 30 per cent of animals and therefore it was not investigated.

The effect of atropine and physostigmine. Atropine (0·5 mg/kg) did not affect the ability of physostigmine to produce glycogenolysis either in the diaphragm or the

TABLE 1. THE EFFECT OF INCREASING DOSES OF PHYSOSTIGMINE ON THE AMOUNT OF GLYCOGEN IN THE DIAPHRAGM AND TRICEPS MUSCLE OF THE RAT

Organ	Controls	Physostigmine treated animals		
		100 μg/kg	200 μg/kg	400 μg/kg
Diaphragm	361·3 ± 5·1 (6)	300 ± 13·4* (4)	238·5 ± 7·7* (4)	188·5 ± 8* (4)
Triceps muscle	522·6 ± 6·3 (6)	507·5 ± 11·8 (4)	499·5 ± 10·3 (4)	445·2 ± 10·9* (4)

The amount of glycogen is expressed in mg%. The numbers indicate the mean value ± S.E.M.
The number of experiments is indicated between brackets.
* $P < 0.001$ in comparison with the controls.

TABLE 2. THE EFFECT OF ATROPINE AND PHYSOSTIGMINE ON THE AMOUNT OF GLYCOGEN IN THE DIAPHRAGM AND TRICEPS MUSCLE OF THE RAT

Treatment	Diaphragm	Triceps muscle
1. Controls	361·3 ± 5·1 (6)	522·6 ± 6·3 (6)
2. Atropine (0·5 mg/kg)	338·7 ± 12 (4)	505·5 ± 7 (4)
3. Atropine (0·5 mg/kg) + Physostigmine (200 μg/kg)	256 ± 5·8 (3)	—
4. Physostigmine (200 μg/kg)	238·5 ± 7·7 (4)	499·5 ± 10·3 (4)
5. Physostigmine (400 μg/kg)	188·5 ± 8 (4)	445·2 ± 10·9 (4)
6. Atropine (0·5 mg/kg) + Physostigmine (400 μg/kg)	215·7 ± 9·1 (4)	461 ± 10 (4)

The amount of glycogen is expressed in mg%. The numbers indicate the mean value ± S.E.M. The number of experiments is indicated in parentheses.

P (Diaphragm) (1:2) not significant
P ,, (1:4) < 0·001
P ,, (1:5) < 0·001
P ,, (3:4) not significant
P ,, (5:6) not significant
P ,, (2:3) < 0·01
P ,, (2:6) < 0·001

P (Triceps) (1:5) < 0·001
P ,, (5:6) not significant

triceps. There was no difference in the glycogenolytic effect between the animals receiving only physostigmine and those treated with both atropine and physostigmine (Table 2).

Atropine by itself did not produce any significant change in the amount of glycogen either in the diaphragm or the triceps muscle.

The effect of propranolol and physostigmine. Propranolol (10 mg/kg) was found to antagonize almost completely the glycogenolytic effect of physostigmine. In the animals pretreated by propranolol physostigmine still produced a small decrease in the amount of glycogen in diaphragm, but this decrease was significantly less than in the animals treated by physostigmine alone (Table 3).

Propranolol by itself did not affect the amount of glycogen in diaphragm.

TABLE 3. THE EFFECT OF PROPRANOLOL AND PHYSOSTIGMINE ON THE AMOUNT OF GLYCOGEN IN DIAPHRAGM OF THE RAT

Treatment	Glycogen (mg%)	P
1. Controls	361·3 ± 5·1 (6)	P(1:3) < 0·001
2. Propranolol (10 mg/kg)	355·8 ± 5·1 (5)	P(1:2) not significant
3. Physostigmine (200 μg/kg)	238·5 ± 7·7 (4)	P(1:4) < 0·01
4. Physostigmine (200 μg/kg) + Propranolol (10 mg/kg)	334·4 ± 3·1 (5)	P(3:4) < 0·001
5. Physostigmine (400 μg/kg)	188·5 ± 8 (4)	P(1:5) < 0·001 P(5:6) < 0·001
6. Physostigmine (400 μg/kg) + Propranolol (10 mg/kg)	328 ± 4·6 (5)	P(1:6) < 0·01 P(2:4) < 0·01 P(2:6) < 0·01

The numbers indicate the mean value ± S.E.M. The number of experiments is indicated in parentheses.

The effect of physostigmine in vitro. In a series of experiments in which strips of diaphragm were incubated *in vitro* in Tyrode solution for 30 min at 36·7°, physostigmine did not affect the amount of glycogen if used in a concentration which roughly corresponded to the concentration which might be expected *in vivo* after injection of the above-mentioned low and medium doses of physostigmine. In ten control experiments glycogen was found in a concentration of 151·3 ± 3·9 mg%. If the diaphragm was incubated for 30 min in a medium containing 5 μg/ml physostigmine, the concentration of glycogen was 146·2 ± 5·6 mg% (five experiments). In a group of five experiments physostigmine was used in a concentration of 10 μg/ml and it produced a decrease of glycogen to 124·4 ± 2·9 mg% ($P < 0·01$). A very high concentration of physostigmine (50 μg/ml) produced a highly significant decrease in the amount of glycogen in diaphragm to 65·6 ± 7·2 mg% (five experiments) ($P < 0·001$).

DISCUSSION

It was shown in the present experiments that physostigmine administered intravenously to rats produced a significant decrease in the amount of glycogen in the diaphragm. This effect of physostigmine was found to be dose-dependent. The range of

doses of physostigmine was roughly the same as that used to produce blood pressure rises in the rat.[8, 9] On the other hand, only a very high dose of physostigmine was able to produce a decrease in the amount of glycogen in the triceps muscle. A difference in the glycogen content of the hypothalamus and some other brain structures in response to physostigmine has already been observed.[2]

Neostigmine, even in a dose which was lethal for some animals, did not affect the amount of glycogen in both diaphragm and triceps muscle. Atropine did not affect the amount of glycogen in these two muscles either. These two findings indicate that a peripheral cholinergic process, implicating muscarinic receptors, most probably does not participate in the glycogenolytic effect of physostigmine in the diaphragm.

Physostigmine has already been known to produce in the rat a general sympathetic activation of the central origin.[8, 10] The consequence of this activation might be either an increase in the blood pressure,[9] or a glycogenolytic effect in the liver[1] and various brain structures.[2] In all these effects an initial activation of central cholinergic processes, which in turn activate adrenergic processes, was postulated.[11] It is therefore possible that the same mechanism also takes place in the mechanism of the glycogenolytic effect of physostigmine in the diaphragm. The adrenergic nature of this effect is further supported by the finding that it was almost completely blocked by the adrenergic beta-blocking agent propranolol. As both the hypertensive response to physostigmine and its glycogenolytic effect in the liver are also present in the adrenalectomized animal[1] it is most probable that, after general sympathetic activation, noradrenaline as sympathetic transmitter is the factor which causes the activation of the processes leading to glycogenolysis in the diaphragm

Both alpha- and beta-adrenergic receptors are said to be implicated in glycogenolysis.[12] In some organs at least, this process seems to be mediated by adrenergic beta-receptors. In both liver and skeletal muscle the glycogenolysis produced by catecholamines or induced by activation of the sympathetic nervous system could be blocked by adrenergic beta-blocking agents.[4, 13, 14] Our experiments indicate that adrenergic beta-receptors also mediate the glycogenolysis in the diaphragm. Assuming that after sympathetic activation by physostigmine the sympathetic transmitter is mainly responsible for glycogenolysis in the diaphragm, the failure of physostigmine to produce (after small and medium doses) glycogenolysis in triceps muscle is in agreement with the finding of Hynie *et al.*[12] that noradrenaline does not influence "muscle glycogen" and lactacidaemia, while producing glycogenolysis in the liver.

In vitro, the concentrations of physostigmine which roughly corresponded to those *in vivo* after low and medium doses used in these experiments, did not significantly affect the amount of glycogen in the diaphragm. Very high concentrations, however, produced a significant decrease in the amount of glycogen in this muscle. These high concentrations might be expected to produce a change in ion fluxes leading to a loss of cellular K and an increase in intracellular Na. These ion changes have been known to inhibit 3,5-phosphodiesterase resulting in a higher rate of glycogenolysis.[15] It is therefore concluded that physostigmine produces glycogenolysis in the rat diaphragm through an activation of adrenergic mechanisms, most probably by a central action. Peripheral cholinergic processes, implicating muscarinic receptors, do not take part in glycogenolysis in the diaphragm.

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