Biochemical Pharmacology, Vol. 20, pp. 1191–1199. Pergamon Press, 1971. Printed in Great Britain

EFFECT OF PHYSOSTIGMINE AND NEOSTIGMINE ON THE GLYCOGEN STORES IN THE HEART AND IN THE ADRENALS

B. B. MRŠULJA and V. M. VARAGIĆ

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

(*Received* 15 *June* 1970; *accepted* 30 *October* 1970)

Abstract—Physostigmine (25–400 μg/kg) was found to produce an increase in the amount of glycogen in the heart atria and heart apex, and at the same time a decrease in the amount of glycogen in the adrenals. Neostigmine (25–50 μg/kg) also produced an increase in the amount of glycogen in the heart atria, but did not affect the amount of glycogen in the adrenals. All these effects were dose-dependent. Atropine was found to block in an equally effective way both the effect of physostigmine in increasing the amount of glycogen in the heart, and its glycogenolytic effect in the adrenals. The effect of neostigmine in increasing the amount of glycogen in the heart atria was also blocked by atropine. Propranolol blocked only the glycogenolytic effect of physostigmine in the adrenals. In 6-hydroxy-dopamine treated rats physostigmine did not produce the glycogenolytic effect in the adrenals. 6-Hydroxy-dopamine itself was found to produce an increase in the amount of glycogen in the adrenals.

PHYSOSTIGMINE has been shown to produce glycogenolysis in various tissues of the rat. Thus, the intravenous injection of physostigmine produced a decrease in the glycogen stores in the liver,[1] in the various structures of the central nervous system,[2] and in the striated muscle.[3] In contrast to physostigmine, neostigmine did not affect the glycogen stores in either of these tissues. The glycogenolytic effect of physostigmine was explained by its activation of adrenergic mechanisms which are known to participate in glycogenolysis. This effect is supposed to be of the central origin and it is probably similar to the hypertensive action of physostigmine in the rat.[4–6]

The adrenals are known as the largest single pool of catecholamines and the heart atria are known to be very rich in sympathetic arborization. Some preliminary data indicated that physostigmine might affect the glycogen stores in the heart atria in a manner which is different from that in the other tissues.[1,7]

In the regulation of heart phosphorylase activity, the sympathetic nervous system has been found to be a factor of fundamental importance.[8] On the other hand, cardiac phosphorylase activity can also be affected by the parasympathetic system.[9] This is best demonstrated when the activity of this system is already elevated. Taking into account that physostigmine can produce a central adrenergic activation and neostigmine can produce a peripheral cholinergic stimulation, it was of interest to investigate the effects of these two substances on the glycogen stores in the heart and in the adrenals of the rat.

MATERIALS AND METHODS

Only male albino rats (190–220 g) were used in these experiments. Standard food and water were allowed to all animals *ad lib.*

Physostigmine salicylate and neostigmine methylsulphate were injected intravenously. These anticholinesterases were allowed to act for 30 min before the animals were sacrificed. Propranolol hydrochloride (10 mg/kg), phenoxybenzamine hydrochloride (5 mg/kg) and atropine sulphate (0·5 mg/kg) were injected intraperitoneally. These blocking agents were allowed to act for 20 min before physostigmine or neostigmine were injected. 6-Hydroxy-dopamine (30 mg/kg) was also injected intravenously.

The animals were sacrificed by means of a specially constructed guillotine. For determination of cardiac glycogen both heart atria were dissected as quickly as possible, within 60 sec after death of the animal. In a separate series of animals a piece of heart apex, weighing about 25 mg, was dissected. Care was taken that the sample is always cut from the same anatomical region. Again in a separate series of animals both adrenals were dissected (because it was impossible to dissect both the heart and adrenals within 60 sec).

Glycogen was estimated according to the method described by Montgomery.[10]

The following substances were used: physostigmine salicylate, neostigmine methylsulphate (Prostigmin "Roche"), propranolol hydrochloride (Inderal "ICI"),* phenoxybenzamine hydrochloride, atropine sulphate and 6-hydroxy-dopamine hydrobromide (Regis Chemical Co.).

RESULTS

The effect of physostigmine. The increasing doses of physostigmine were found to affect quite differently the glycogen stores in the heart atria and in the adrenals. The intravenous injection of physostigmine (25–400 μg/kg) was found to produce a dose-dependent increase in the amount of glycogen in the heart atria, and a dose-dependent decrease in the amount of glycogen in the adrenals. All these changes were statistically highly significant in comparison to the control values of glycogen either in the heart atria or in the adrenals ($P < 0{\cdot}001$). There was a linear relationship between the doses of physostigmine (on the log scale) and the percentage increase or decrease in the amount of glycogen (Figs. 1 and 2).

The other characteristic of the effect of physostigmine on the glycogen stores in the heart atria was an inverse relationship between the doses and the effect, i.e. the small doses produced a large increase in the amount of glycogen, whereas large doses of physostigmine produced a smaller, but still highly significant, increase in the amount of glycogen (Fig. 1). On the other hand, the glycogenolytic effect of physostigmine in the adrenals was directly proportional to the doses used, i.e. the small doses produced a small glycogenolytic effect and the large doses produced a strong glycogenolysis (Fig. 2).

The increasing doses of physostigmine (50–200 μg/kg) were also found to produce an increase in the amount of glycogen in the heart apex, but—contrary to the heart atria—this increase was directly proportional to the doses used. A linear relationship was found if the doses (on the log scale) were plotted against percentage increase in the glycogen concentration (Fig. 3).

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

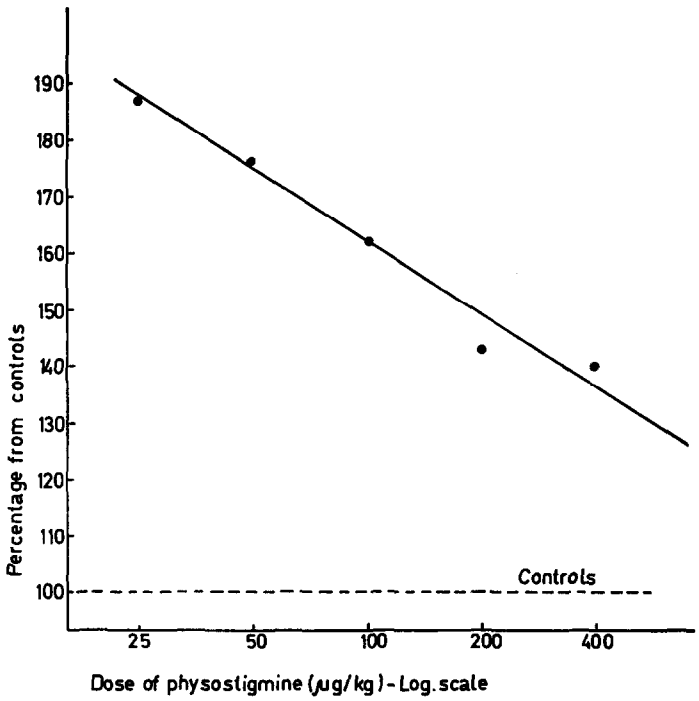

FIG. 1. The effect of increasing doses of physostigmine on the amount of glycogen in the heart atria.

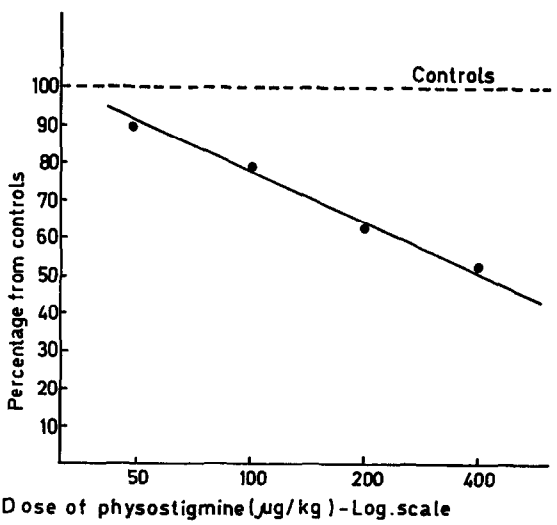

FIG. 2. The effect of increasing doses of physostigmine on the amount of glycogen in the adrenals.

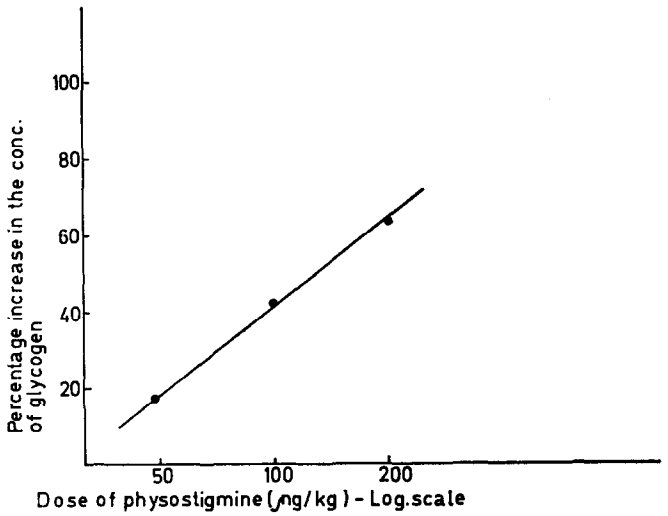

FIG. 3. The effect of increasing doses of physostigmine on the amount of glycogen in the heart apex.

The effect of neostigmine. Neostigmine was injected in doses of 25 and 50 μg/kg. Both these doses were lethal for some of the animals injected. These doses of neostigmine were found to produce an increase in the amount of glycogen in the heart atria. In five animals treated with 25 μg/kg the amount of glycogen in the heart atria was 319 ± 7·8 mg% (mean ± S.E.M., one animal died out of six injected). In another group of five animals the dose of 50 μg/kg neostigmine increased the amount of glycogen to 405 ± 10·0 mg% (three animals died out of eight injected). In comparison with the controls (233 ± 7·1 mg%, three animals), the increase in the concentration of glycogen was statistically highly significant ($P < 0{\cdot}001$).

Atropine (0·5 mg/kg intraperitoneally) was found to block the effect of neostigmine. Thus, in a group of four animals treated with both atropine and neostigmine (25 μg/kg) the concentration of glycogen was 237 ± 7·7 mg%. This value is practically the same as that in the control animals.

On the contrary, neostigmine did not affect the amount of glycogen in the adrenals. Thus, in two groups of animals treated with the above-mentioned doses of neostigmine, the concentration of glycogen was 364 ± 5·3 mg% (five animals) and 370 ± 7·2 mg% (five animals). There was no statistical difference between these values and that in the control animals (373 ± 4·3 mg%, three animals). The mortality rate in these two groups of animals was 28 and 44, indicating the toxicity of the doses used.

The effect of propranolol. Propranolol (10 mg/kg intraperitoneally) did not affect the action of physostigmine in increasing the amount of glycogen in the heart atria. There was no difference in the concentration of glycogen in the heart atria between the animals injected with the increasing doses of physostigmine only, and those injected with both physostigmine and propranolol (Table 1).

On the other hand, the same dose of propranolol was found to block completely the glycogenolytic effect of physostigmine in the adrenals. There was no statistically significant difference between the amount of glycogen in the animals treated with both

TABLE 1. THE EFFECT OF PROPRANOLOL (10 mg/kg INTRAPERITONEALLY) AND OF THE INCREASING DOSES OF PHYSOSTIGMINE ON THE CONCENTRATION OF GLYCOGEN IN THE HEART ATRIA OF THE RAT

Treatment	Glycogen (mg%)	P
1. Controls	240 ± 5·2 (4)	P(1:2) not significant
2. Propranolol (10 mg/kg)	237 ± 4·3 (3)	P(1:3) < 0·001
3. Physostigmine salicylate (25 μg/kg)	451 ± 5·1 (5)	P(1:4) < 0·001
4. Physostigmine salicylate (50 μg/kg)	423 ± 6·5 (4)	P(1:5) < 0·001
5. Physostigmine salicylate (100 μg/kg)	390 ± 7·3 (4)	P(1:6) < 0·001
6. Physostigmine salicylate (200 μg/kg)	342 ± 5·1 (4)	P(1:7) < 0·001
7. Physostigmine salicylate (400 μg/kg)	337 ± 5·0 (4)	
8. Propranolol (10 mg/kg) + physostigmine salicylate (50 μg/kg)	413 ± 6·8 (4)	P(4:8) not significant
9. Propranolol (10 mg/kg) + physostigmine salicylate (100 μg/kg)	381 ± 2·2 (4)	P(5:9) not significant
10. Propranolol (10 mg/kg) + physostigmine salicylate (200 μg/kg)	338 ± 4·2 (4)	P(6:10) not significant
11. Propranolol (10 mg/kg) + physostigmine salicylate (400 μg/kg)	306 ± 9·2 (3)	P(7:11) not significant

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

TABLE 2. THE EFFECT OF PROPRANOLOL (10 mg/kg INTRAPERITONEALLY) ON THE GLYCOGENOLYTIC EFFECT OF PHYSOSTIGMINE IN THE ADRENALS OF THE RAT

Treatment	Glycogen (mg%)	P
1. Controls	373 ± 8·7 (4)	
2. Propranolol (10 mg/kg)	378 ± 7·2 (3)	P(1:2) not significant
3. Physostigmine salicylate (50 μg/kg)	337 ± 6·6 (4)	P(1:3) < 0·05
4. Physostigmine salicylate (100 μg/kg)	297 ± 6·0 (4)	P(1:4) < 0·001
5. Physostigmine salicylate (200 μg/kg)	238 ± 6·9 (4)	P(1:5) < 0·001
6. Propranolol (10 mg/kg) + physostigmine salicylate (50 μg/kg)	373 ± 8·7 (4)	P(1:6) not significant
7. Propranolol (10 mg/kg) + physostigmine salicylate (100 μg/kg)	365 ± 7·8 (4)	P(1:7) not significant
8. Propranolol (10 mg/kg) + physostigmine salicylate (200 μg/kg)	340 ± 7·3 (4)	P(1:8) not significant

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

physostigmine and propranolol, and the amount of glycogen in the controls (Table 2).

The effect of phenoxybenzamine. It has been already found that the action of adrenaline on the glycogen synthetase system of heart, in contrast to skeletal muscle, is to increase the proportion of this enzyme in the active form I.[21,22] In order to check up whether adrenergic alpha receptors might be involved in the action of physostigmine in increasing the amount of glycogen in the heart atria, phenoxybenzamine (5 mg/kg intraperitoneally) was used in a separate series of experiments. It was found that this substance did not antagonize the effect of physostigmine, either. Thus, in three animals treated with phenoxybenzamine only, the amount of glycogen in the heart atria and in the heart apex were 288 ± 4·3 mg% and 196 ± 4·3 mg%, respectively. These values were very close to those found in the control non-treated animals. Physostigmine salicylate (100 μg/kg) was found to increase the amount of glycogen in the heart atria and in the heart apex to 386 ± 10·0 mg% (three animals) and to 293 ± 8·8 mg% (three animals), respectively. In animals treated by both phenoxybenzamine and physostigmine, the amount of glycogen in the heart atria and in the heart apex was 395 ± 7·6 mg% and 286 ± 10·0 mg%, respectively (three animals in each group). There was no difference between these values and those obtained in animals treated by physostigmine only.

The effect of atropine. Atropine (0·5 mg/kg intraperitoneally) was found to block completely both the effect of physostigmine in increasing the amount of glycogen in the heart atria, and the action of physostigmine in decreasing the amount of glycogen in the adrenals. There was no statistically significant difference between the amount of glycogen in the animals treated with both atropine and physostigmine, and the amount of glycogen in the controls (Table 3).

TABLE 3. THE EFFECT OF ATROPINE (0·5 mg/kg INTRAPERITONEALLY) ON THE CHANGES IN THE GLYCOGEN STORES IN THE HEART ATRIA AND IN THE ADRENALS PRODUCED BY INTRAVENOUS INJECTION OF PHYSOSTIGMINE IN THE RAT

Treatment	Glycogen (mg%) A. Heart atria	B. Adrenals	P
1. Controls	240 ± 5·2 (4)	373 ± 8·7 (4)	
2. Atropine sulphate (0·5 mg/kg)	243 ± 6·0 (3)	373 ± 4·7 (3)	2A:6A not significant
3. Physostigmine salicylate (50 μg/kg)	423 ± 6·5 (4)	337 ± 6·6 (4)	2B:6B not significant
4. Physostigmine salicylate (100 μg/kg)	390 ± 7·3 (4)	297 ± 6·0 (4)	2A:7A not significant
5. Physostigmine salicylate (200 μg/kg)	342 ± 5·1 (4)	238 ± 6·9 (4)	2B:7B not significant
6. Atropine sulphate (0·5 mg/kg) + physostigmine salicylate (50 μg/kg)	236 ± 10·1 (3)	371 ± 7·2 (3)	2A:8A not significant
7. Atropine sulphate (0·5 mg/kg) + physostigmine salicylate (100 μg/kg)	226 ± 4·3 (3)	365 ± 8·6 (3)	2B:8B not significant
8. Atropine sulphate (0·5 mg/kg) + physostigmine salicylate (200 μg/kg)	232 ± 9·4 (3)	371 ± 11·6 (3)	

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

The effect of 6-*hydroxy-dopamine.* In order to check up the adrenergic nature of the effect of physostigmine in the adrenals, a separate series of experiments was performed in which the rats were previously treated by 6-hydroxy-dopamine (30 mg/kg intravenously). It has been already known that, depending on the dose, 6-hydroxy-dopamine can produce either a slow developing block of the hypertensive effect of physostigmine, or an immediate abolition of the physostigmine response.[23]

In the present experiments 6-hydroxy-dopamine itself was found to produce an increase in the amount of glycogen in the adrenals. In a group of five rats treated by 6-hydroxy-dopamine, the amount of glycogen in the adrenals was 450 $\pm$ 6·8 mg%. In comparison with the untreated four controls (373 $\pm$ 8·7 mg%) this value was significantly higher ($P < 0{\cdot}001$). In a group of five rats previously treated by 6-hydroxy-dopamine and then by physostigmine (100 μg/kg) the amount of glycogen in the adrenals was 431 $\pm$ 5·7 mg%. This value is hardly different from the "6-hydroxy-dopamine controls" ($P < 0{\cdot}05$), but it is highly significantly different from the value (297 $\pm$ 6·0 mg%) obtained with the same dose of physostigmine in the untreated rats ($P < 0{\cdot}001$).

The effect of physostigmine in vitro. Physostigmine (5 μg/ml) did not affect the amount of glycogen in the isolated adrenals *in vitro.* In this series of three experiments both adrenals were incubated in Krebs-bicarbonate solution (pH 7·4) at 36·7° for 10 min. The amount of glycogen in the control group of three experiments was 188 $\pm$ 16·4 mg%. In another group of three experiments, in which physostigmine was added to the incubating medium, the amount of glycogen was almost the same as in the control group—180 $\pm$ 13·2 mg%.

On the other hand, physostigmine (5 μg/ml) was found to produce and increase in the amount of glycogen in the isolated heart atria and heart apex *in vitro.* In three control experiments, in which both heart atria were incubated in the same manner as in the previous experiments, the amount of glycogen was 130 $\pm$ 8·1 mg%. In the presence of physostigmine (three experiments) the amount of glycogen was significantly increased to 163 $\pm$ 6·6 mg% ($P < 0{\cdot}02$). Similar results were obtained with the isolated heart apex. In three control experiments the amount of glycogen was 83 $\pm$ 4·3 mg%, whereas in the presence of physostigmine (three experiments) it rose to 113 $\pm$ 4·3 mg% ($P < 0{\cdot}005$).

DISCUSSION

The results of the present experiments indicate that physostigmine affected the glycogen stores in the heart and in the adrenals in quite opposite directions: it produced an *increase* in the amount of glycogen both in the heart atria and in the heart apex, and it caused a *decrease* in the amount of glycogen in the adrenals. All these effects were found to be dose-dependent. The effect of physostigmine in increasing the amount of glycogen in the heart apex and its glycogenolytic effect in the adrenals were directly proportional to the doses used. On the other hand, the effect of physostigmine in increasing the amount of glycogen in the heart atria was found to be inversely proportional to the doses used, i.e. the small doses of physostigmine produced a large increase in the amount of glycogen, whereas the large doses of physostigmine produced a small but still significant increase in the amount of glycogen. All the above-mentioned effects were obtained with the doses of physostigmine which have been known to produce hypertensive response in the rat.[1,6,10]

Neostigmine, in doses which were lethal for some animals, did not affect the amount of glycogen in the adrenals. This finding is in agreement with our previous results in which neostigmine did not change the glycogen stores in the liver,[1] diaphragm[3] and in the various parts of the central nervous system.[2] Contrary to these findings, neostigmine was found in the present experiments to produce a dose-dependent increase in the amount of glycogen in the heart atria. In contrast to physostigmine, the effect of neostigmine was directly proportional to the doses used.

The different mechanisms participating in the effects of physostigmine on the glycogen stores in the heart and in the adrenals can be seen from different responses to propranolol and atropine. Atropine was found to block in an equally effective way both the effect of physostigmine in increasing the amount of glycogen in the heart, and its glycogenolytic effect in the adrenals. The effect of neostigmine in increasing the amount of glycogen in the heart atria was also blocked by atropine. On the other hand, propranolol blocked only the glycogenolytic effect of physostigmine in the adrenals, but it did not affect the action of physostigmine in increasing the amount of glycogen in the heart atria and heart apex. Phenoxybenzamine did not affect this action of physostigmine, either. The glycogenolytic effect of physostigmine in the adrenals was blocked by pretreatment with 6-hydroxy-dopamine.

Physostigmine has been already known to produce a general sympathetic activation in the rat[4–6] which can produce either an increase in the blood pressure, or a glycogenolytic effect.[1–3] An initial cholinergic process leading to the adrenergic activation has been postulated.[11] The results of the present experiments with the adrenals are in agreement with those previously obtained with liver and diaphragm. Most probably, a general sympathetic activation of the central origin participates in the glycogenolytic effect of physostigmine in the adrenals. This is also supported by the finding that the glycogenolytic effect of physostigmine in the adrenals was blocked in the animals pretreated with 6-hydroxy-dopamine. Adrenergic mechanisms in the glycogenolysis produced by physostigmine in the adrenals are mediated by beta-receptors. It has been already known that glycogenolysis in liver and skeletal muscle produced by catecholamines or by higher activity of the sympathetic nervous system can be blocked by adrenergic beta-blocking agents.[12–14]

The participation of cholinergic processes both in the effect of physostigmine in increasing the amount of glycogen in the heart and in decreasing the amount of glycogen in the adrenals is indicated by the blocking action of atropine in both cases. The blocking action of atropine on the glycogenolysis in the adrenals induced by physostigmine is presumably central, whereas its blocking action on the effect of physostigmine in inducing an increase in the amount of glycogen in the heart is probably peripheral. This is supported by the finding that both the effects of physostigmine and neostigmine in the heart are blocked by atropine, and also by the finding that physostigmine affects the glycogen stores in the heart *in vitro*. Neostigmine as a quaternary substance does not penetrate the central nervous system and it probably acts only peripherally.

The action of the parasympathetic system and of the cholinergic drugs on the cardiac phosphorylase has not been studied extensively and it is not well understood.[13] Acetylcholine has been shown to diminish the glycogen content in the perfused heart[15] and to depress the synthesis of cyclic AMP in the cell-free heart preparations.[16] In the open-chest rat preparation injection of acetylcholine or electrical stimulation of the

vagus nerve decreased heart phosphorylase *a* activity and antagonized phosphorylase stimulation produced by the ganglion-stimulating agent McNeil-A-343.[9] The results of our present experiments in which anticholinesterases were found to increase the amount of glycogen in the heart atria both *in vivo* and *in vitro* can at least partly be explained by a decrease in heart phosphorylase *a* activity. Meanwhile, it is possible—but not proved by the present experiments—that the observed effect might be due to induction of glycogen synthetase. Such an effect has been shown for insulin and glucocorticoids.[17–20]

The question why physostigmine activated an adrenergic process in the adrenals, and at the same time activated a cholinergic process in the heart, is not solved by the present experiments. This may depend on the functional specificity of these two organs. More research is necessary in order to clear the complexity of the glycogen metabolism in the heart.

REFERENCES

1. V. Varagić, M. Terzić and B. Mršulja, *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.* **258**, 229 (1967).
2. B. Mršulja, M. Terzić and V. M. Varagić, *J. Neurochem.* **15**, 1329 (1968).
3. B. B. Mršulja, M. Terzić and V. M. Varagić, *Biochem. Pharmac.* **19**, 2125 (1970).
4. V. Varagić, *Br. J. Pharmac.* **10**, 349 (1955).
5. V. Varagić and M. Krstić, *Pharmac. Rev.* **18**, Part I, 799 (1966).
6. V. M. Varagić, M. Žugić and D. Kentera, *Iugoslav. Physiol. Pharmac. Acta* **5**, 59 (1969).
7. B. Mršulja and M. Terzić, *C. r. Soc. Biol.* **161**, 2434 (1967).
8. N. Naugaard and M. E. Hess, *Pharmac. Rev.* **18**, Part I, 197 (1966).
9. M. E. Hess, J. Shanfeld and N. Haugaard, *J. Pharmac.* **135**, 191 (1962).
10. R. Montgomery, *Archs Biochem. Biophys.* **67**, 378 (1957).
11. V. M. Varagić, T. Kažić and N. Rosić, *Iugoslav. Physiol. Pharmac. Acta* **4**, Suppl. 1, 113 (1968).
12. B. B. Brodie, J. I. Davies, S. Hynie, G. Krishna and B. Weiss, *Pharmac. Rev.* **18**, Part I, 273 (1966).
13. N. Haugaard and M. E. Hess, *Pharmac. Rev.* **17**, 27 (1965).
14. C. J. Estler, O. Strubelt and H. P. T. Ammon, *Pflügers Arch. ges. Physiol.* **289**, 227 (1966).
15. N. H. Vincent and S. Ellis, *J. Pharmac.* **139**, 60 (1963).
16. F. Murad, Y. M. Chi, T. W. Rall and E. W. Sutherland, *J. biol. Chem.* **237**, 1233 (1962).
17. H. G. Sie, A. Hablanian and W. H. Fishman, *Biochem. J.* **102**, 103 (1967).
18. D. F. Steiner and J. King, *J. biol. Chem.* **239**, 1292 (1964).
19. H. de Wulf and H. G. Hers, *Europ. J. Biochem.* **2**, 57 (1967).
20. G. Senft, *Naunyn Schmiedebergs Arch. Pharmak. exp. Path.* **259**, 117 (1968).
21. J. Himms-Hagen, *Pharmac. Rev.* **19**, 367 (1967).
22. J. R. Eilliamson, *Pharmac. Rev.* **18**, 205 (1966).
23. V. M. Varagić and T. Kažić, *Neuropharm.* **9**, 97 (1970).