The Influence of Antistine on Glycogenolytic Effect of some Biogenic Amines in Rat Brain Slices

The formation of cyclic 3′, 5′-adenosine-monophosphate (CAMP) in slices of brain tissue has been shown to be stimulated by such neurotransmitters as adrenaline, noradrenaline, histamine and serotonin[1], but the relative potencies of these agents vary considerably from species to species[2]. The brain has been shown to contain the highest activities of both adenyl cyclase[3] and CAMP phosphodiesterase[4] in the mammalian tissues that have been investigated. Although it was well established that adenyl cyclase activity can be enhanced by various putative transmitters, it was recently shown that the mechanism of in vivo activation may differ from in vitro findings[5]. The data obtained suggested that monoamines, but not acetylcholine, can significantly stimulate adenyl cyclase activity of rat cerebral cortex in vivo[5].

The control of glycogen content is a very good model for investigation of the action of CAMP in the central nervous system, because the levels of CAMP control of both glycogen phosphorylase and glycogen systhethase[6]. Recently it was shown that putative neurotransmitters, such as adrenaline, noradrenaline, dopamine, serotonin and histamine, produced glycogenolysis in slices of rat brain (cortex, caudate and thalamus)[7,8]. On the other hand, the effect of dopamine and noradrenaline could be blocked with classical β-adrenergic blockers, but not with α-adrenergic blocking agents, neither of these blockers could prevent the glycogenolytic effects of serotonin and histamine, as well as of CAMP and cyclic N-2-*O*-dibutyryl-adenosine-3′, 5′-monophosphate (db-CAMP)[9]. The results obtained suggested that in rat brain the separate type of receptors for different biogenic amines possibly exist, especially for noradrenaline and histamine.

In order to confirm the hypothesis we investigated the influence of potent antihistaminic agent antistine on glycogenolytic effect of noradrenaline, dopamine, serotonin, as well as of CAMP and db-CAMP.

The experiments were carried out on adult male Wistar rats. Brain slices were prepared according to the method already described[10] and were allowed 10 min in saline[10] at 37 °C. Antistine (2 mg/kg) was injected i.p. 30 min before the animals were sacrificed. The brain slices were incubated in the presence of noradrenaline (10^{-4} μM/ml), dopamine (10^{-4} μM/ml), dopamine (10^{-4} μM/ml), histamine (10^{-4} μM/ml), serotonin (10^{-4} μM/ml), CAMP (10^{-3} μM/ml) and db-CAMP (10^{-3} μM/ml) and after 10 min glycogen was extracted[11] and estimated[12] from the brain tissue.

The data obtained show that antistine prevented the glycogenolytic effect of histamine in vitro, but not that of noradrenaline, dopamine and serotonin, neither that of CAMP and db-CAMP (Table).

ROBISON et al.[13] postulated that adenyl cyclase could be an adrenergic receptor and suggested that both α- and β-receptors were regulatory subunits of adenyl cyclase. In view of recently published data in the cerebral cortex, the existence of 2 interacting regulatory units governing adenyl cyclase activity was indicated: one for adenosine and one for biogenic amines[14]; evidence for separate regulatory receptors for histamine and noradrenaline in rabbit cerebellum and cerebelar cortex has also been reported[15]. The existence of compartmentalized pool of adenine nucleotides serving as precursors for CAMP which is regulated in a synergistic manner by separate 'receptors' for adenosine, histamine and either noradrenaline or serotonin was indicated[14].

Our recently obtained results[8,9] indicated that in cortex, caudate and thalamus of rat brain, β-adrenergic regulatory unit of adenyl cyclase is responsible for the level of CAMP and activity of glycogen phosphorylase. On the other hand, another type of regulatory unit responsible for action of histamine and perhaps for serotonin has been suggested[9,16]. The data also suggested that α-adrenergic regulatory unit most probably is not involved in the process of glycogenolysis in the rat brain tissue.

Histamine was shown to be the most active stimulator of CAMP although adrenaline and noradrenaline were both clearly active[1,15]. On the other hand, histamine produced the most significant fall of glycogen content in rat brain slices[8]. The areas shown to contain significant levels

[1] S. KAKIUCHI and T. W. RALL, Molec. Pharmac. *4*, 379 (1968).
[2] J. FORN and G. KRISHNA, Fedn. Proc. *29*, 480 (1970).
[3] E. W. SUTHERLAND, T. W. RALL and T. MANON, J. biol. Chem. *237*, 1220 (1962).
[4] R. W. BUTCHER and E. W. SUTHERLAND, J. biol. Chem. *237*, 1244 (1962).
[5] W. S. CHOU, A. K. S. Ho and H. H. LOH, Nature, New Biol. *233*, 280 (1971).
[6] J. HIMMS-HAGEN, Pharmac. Rev. *9*, 267 (1967).
[7] B. B. MRŠULJA and L. M. RAKIĆ, J. Neurochem. *17*, 821 (1970).
[8] B. B. MRŠULJA, Experientia *28*, 1067 (1972).
[9] B. B. MRŠULJA, Experientia *28*, 1072 (1972).
[10] R. RODNIGHT and H. MCILWAIN, Biochem. J. *57*, 649 (1954).
[11] F. N. LEBARON, Biochem. J. *61*, 80 (1955).
[12] R. MONTGOMERY, Arch. Biochem. *67*, 378 (1957).
[13] G. A. ROBISON, R. W. BUTCHER and E. W. SUTHERLAND, Ann. N. Y. Acad. Sci. *139*, 703 (1976).
[14] M. HUANG, H. SHIMIZU and J. DALY, Molec. Pharmac. *7*, 155 (1971).
[15] S. KAKIUCHI and T. W. RALL, Molec. Pharmac. *4*, 367 (1968).
[16] H. CHASIN, I. RIVKIN, F. MAMRAK, S. G. SAMANIEGRO and S. A. HESS, J. biol. Chem. *246*, 3037 (1971).

In vitro effects of noradrenaline, dopamine, histamine, serotonin, CAMP and db-CAMP on glycogen concentration in brain of rat treated before with antistine (2 mg/kg)

Treatment of the tissue	Brain slices Cortex	Caudate	Thalamus
1. Controls	27.4 ± 1.3	44.0 ± 1.4	16.0 ± 1.1
2. Noradrenaline (10^{-4} μM/ml)	20.9 ± 0.9[b]	23.1 ± 1.4[a]	7.1 ± 1.1[a]
3. Dopamine (10^{-4} μM/ml)	22.1 ± 1.1[b]	27.4 ± 1.3[b]	9.8 ± 1.0[b]
4. Histamine (10^{-4} μM/ml)	30.5 ± 1.4	42.5 ± 1.3	17.8 ± 1.2
5. Serotonin (10^{-4} μM/ml)	15.4 ± 1.4[a]	23.3 ± 1.3[a]	8.0 ± 1.1[a]
6. CAMP (10^{-3} μM/ml)	20.1 ± 1.4[b]	29.5 ± 1.4[b]	9.5 ± 1.4[b]
7. db-CAMP (10^{-3} μM/ml)	10.4 ± 1.6[b]	19.5 ± 1.3[a]	4.5 ± 1.1[a]

[a] $p < 0.01$ in comparison with the controls. [b] $p < 0.05$ in comparison with the controls. The amount of glycogen is expressed in mg/100 ml of tissue. The numbers indicate the mean value of 5 experiments ± S.E.M.

of histamine[17–19] can increase the levels of CAMP in the slices of brain in the presence of histamine[1].

Classicyl β-adrenergic blocking agents were potent blockers of noradrenaline-stimulated CAMP accumulation in the cerebellum of the guinea-pig[16], as well as the blockers of glycogenolytic influence of noradrenaline and dopamine[9], but not of histamine[9], while classical α-adrenergic blocking agents had no ability to block the phenomena mentioned[9,16]. On the other hand, anti-histaminic agents blocked the accumulation of CAMP by histamine[16]; the results of this work show that antistine also prevented the glycogenolytic influence of histamine, but not that of other tested monoamines, neither of CAMP or db-CAMP. Therefore it seems apparent, that receptors for histamine and noradrenaline and/or serotonin in cerebral cortex, caudate and thalamus of rat are separate and that some blockers are capable of blocking one site without having an effect on the other. The data obtained suggested that histamine by accumulation of CAMP produces glycogenolysis in rat brain and that there must be different subunits of adenyl cyclase for histamine and other monoamines which are involved in the process of glycogenolysis in rat cerebral cortex, caudate and thalamus.

Résumé. On a démontré que l'antistine empêche in vitro l'effet glycogénolytique de l'histamine, bien qu'elle n'ait pas d'influence sur les actions glycogénolytiques de la noradrénaline, de la dopamine, de la sérotonine, du 3′,5′-AMP cyclique et de son dérivé dibutirique. Ces résultats suggèrent l'existence de récepteurs particuliers pour les amines biogénique dans le tissue du cortex, du nucleus caudatus et du thalamus. De ces résultats on a conclu que, dans le cerveau du rat, l'adénocyclase pourrait être le récepteur histaminique.

B. B. Mršulja

Institute of Biochemistry, Laboratory for Neurochemistry, Faculty of Medicine, Yu-11001 Beograde 7 (Yugoslavia), 19 June 1972.

[17] M. Medina and P. A. Shore, biochem. Pharmac. *15*, 1627 (1966).
[18] H. Green and R. W. Erickson, Arch. int. Pharmacodyn. Thér. *166*, 121 (1967).
[19] J. P. Green, *Handbuch für Neurochemie 4*, 221 (1970).

Axoplasmic Organelles: Quantitative Differences between Ventral and Dorsal Root Fibres of the Rat[1,2]

Based on electro-physiological investigations of single peripheral nerve fibers, several parameters for discriminating motor and sensory fibers have been described[3]. Morphology has so far no means for distinguishing different types of nerve fibres within a mixed nerve, except within certain limits by cholinesterase histochemistry[4]. The results presented below suggest a new possibility for differentiating motor and sensory components in peripheral nerves by means of electron microscopy.

In the present investigation, the contents of neuroplasmic organelles (mitochondria, axoplasmic reticulum, neurotubules, and neurofilaments) in comparable caliber classes of rat ventral and dorsal root fibres have been determined by morphometric methods[5]. The statistical significance of the results has been examined by a *t*-test. The demand for the use of nerve fibers of comparable diameters is based on the fact that, according to previous investigations[6,7], the relative number of axon organelles is dependent on fiber size.

In accordance with these previous results[7], the amount of *axonal organelles* was found *related to the axon diameter* as follows (see Table and Figure 1): The percentual amount of mitochondria and axoplasmic reticulum of the cross-sectional area of an axon is higher in small diameter nerve fibres. Likewise, the number of neurotubules related to cross-sectional area is higher in the small nerve fibres of both roots. However, the density of the neurofilaments in areas devoid of other organelles is rather constant.

In addition we found regular *differences between ventral and dorsal root fibres* of the same caliber class:

There are significantly higher amounts of neurotubules and of axoplasmic reticulum in the ventral root fibres than in the dorsal root fibres of comparable size (Figures 1c and 1b).

Less consistent results were found with respect to axoplasmic mitochondria. As shown in Figure 1a, the amount of mitochondria in small ventral root fibres is higher than in small dorsal root fibres, but the reverse situation is seen in the large fibres. The cross-sectional

Morphometry of ventral and dorsal root fibres of the rat

	Very small Dorsal root fibres	Small			Large		
		Ventral root fibres (A-γ)	sign.	Dorsal root fibres	Ventral root fibres (A-α)	sign.	Dorsal root fibres
n	30	30		30	30		30
Cross sectional area of axon in μm^2 (ACS)	3.55 ± 0.27	10.05 ± 0.77	ns	10.98 ± 0.69	51.49 ± 2.03	++	41.85 ± 2.12
Mitochondria (% of total ACS)	2.24 ± 0.39	1.68 ± 0.21	+	1.01 ± 0.16	0.51 ± 0.04	+	0.70 ± 0.06
Axoplasmic reticulum (% of total ACS)	1.99 ± 0.35	2.39 ± 0.20	+	1.71 ± 0.18	1.27 ± 0.05	+++	0.89 ± 0.06
Neurotubules (number/μm^2 of ACS)	16.24 ± 1.12	17.04 ± 0.86	+	14.13 ± 0.70	10.50 ± 0.27	+++	7.64 ± 0.29
Neurofilaments (number/μm^2 of ACS)	149 ± 4.4	143 ± 3.2	++	161 ± 4.2	141 ± 2.6	++	155 ± 2.8

The values give the mean and the standard deviation of the mean. The values of comparable caliber classes of ventral and dorsal root fibres were statistically examined by the Student *t*-test. sign., significance; ns, not significant; +, $p < 0.05$; ++, $p < 0.01$; +++, $p < 0.001$.