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EFFECT OF GABA, MUSCIMOL AND PICROTOXIN GIVEN IN THIRD VENTRICLE ON SERUM CHOLINESTERASES AND MONOAMINE OXIDASE AND ON PLASMA SUCCINIC DEHYDROGENASE IN RATS*

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Abstract : Effect of injection in third ventricle of GABA, the GABA agonist muscimol, and the GABA antagonist picrotoxin on the activities of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and monoamine axidase (MAO) in serum and succinic dehydrogenase (SDH) in plasma has been studied. Surprisingly, the AChE, BuChE, MAO and SDH enzymes activity were inhibited by GABA and muscimol, while they were enhanced by picrotoxin.

Key words : GABA Muscimol Picrotoxin AChE
BuChE MAO SDH

INTRODUCTION

The hypothalamus surrounds the third ventricle (III-V) and contains large amounts of gamma-aminobutyric acid (GABA) a well known inhibitory neurotransmitter, receptors for which have been classified as GABA-A and GABA-B [1]. In most of the experiments GABA-A type of receptor has been studied, since it is sensitive to the classical GABA-A agonist muscimol and to the antagonist picrotoxin [2]. Earlier we have reported that GABA and muscimol while injected into III-V inhibited the enzymes acetylcholinestress (AChE), butyrylcholinesterase (BuChE), monoamine oxidase (MAO) and succinic dehydrogenase (SDH), on the contrary picrotoxin had enhanced these enzymes in the hypothalamic nuclei [3, 4]. The present study designed to see the effect of such injections on serum AChE, BuChE and MAO, and plasma SDH activity. A preliminary account of changes in serum MAO has been given previously [5].

METHODS

Male albino rats (150-200 g) maintained at 22°±2°C were anaesthetized with nembutal^{R} (45 mg/kg, ip), and under aseptic conditions a 23-guage stainless steel guide cannula implanted in III-V [6] for use after 7 days. Continuous oozing of cerebrospinal fluid from the tip of cannula was observed, if mandril was removed, certified its placement in III-V and only such rats were used. Before the day of experimentation, rats were implanted with chronic jugular catheters

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(0.0377 mm o.d.×0.02 mm i.d., polyethylene tubing) [7] for withdrawal of serial blood samples and they were individually housed. Drugs were injected in III-V using a 31 g Hamilton microsyringe. Doses used were : GABA (Sigma, Co., 5 μg/2 μl) muscimol (Ciba Geigy, 5 μg/2 μl) and picrotoxin (Sisco Labs., 2.5 μg/2 μl). Jugular blood samples (0.2 ml/sample) were taken after 1, 15, 30, 45, 60 and 120 min.

The data are presented as the changes in enzyme activities from the basal values (Values immediately taken before the administration of drugs in III-V).

Enzymes estimated in serum were AChE [8], BuChe [8] and MAO [9]. Plasma SDH was estimated according to the method by Kun and Abood [10]. Protein was estimated by the method of Lowry *et al*, [11[.

All data was subjected to analysis of variance (ANOVA). All multiple comparison with a basal value were done by ANOVA followed by Duncan's test. Differences with $p<0.05$ were considered statistically significant.

RESULTS

There was signifiant inhibition in the activities of all the enzymes after III-V injection of GABA (Table I). The inhibition in AChE was significant during 15-45 min and activity returned to basal level at 120 min. In the case of BuChE, decrease was significant, value being about 35% of the basal value, at 45 min and remained low for 120 min. An immediate, significant decrease was observed also in MAO activity, which became more pronounced by 60 min. A significant decrease in SDH enzyme activity was also observed, upto 60 min (60% of the basal value) returned towards basal value by 120 min. Generally, the effect on cholinesterases was less pronounced as compared to that on MAO and SDH.

Even with GABA specific receptor agonist, muscimol, a similar decrease was obtained in AChE and BuChE activities (maximum changes, 45 min;

TABLE I : Effect of third ventricle GABA on serum acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and monoamine oxidase (MAO), and plasma succinic dehydrogenase (SDH) in male rats.

	AChE+	*BuChE*+	*MAO*++	*SDH*+++
Control	4.81±0.13 (100)	3.53±0.09 (100)	0.339±0.005 (100)	80.69±2.06 (100)
Post-injection				
1 min	4.45±0.20 (92)	3.55±0.13 (101)	0.332±0.004 (95)	84.73±4.09 (105)
15 min	3.96±0.04* (82)	3.17±0.16* (90)	0.287±0.005* (85)	73.80±1.20* (91)
30 min	3.55±0 11* (74)	3.05±0.08* (86)	0.268±0.004* (79)	39.55±1.23* (49)
45 min	3.29±0.14* (68)	2.27±0.06* (64)	0.238±0.003* (70)	35.44±2.26* (44)
60 min	4.29±0.09* (89)	2.31±0.07* (65)	0.221±0.004* (65)	31.83±2.58* (39)
120 min	4.67±0.15 (97)	2.54±0.09* (72)	0.314±0.007 (93)	66.05±1.46* (82)

Values are Mean±SEM for n of 4-6 : parentheses, % of control.

Values are compared by one-way analysis of variance and then means are compared by Duncan's test (*$P<0.05$). +, units/mg protein, ++, μmoles of 4 - hydroxyquinoline/90 min/mg protein, +++ μg of TTC reduced/60 min/mg protein.

return to the basal level 120 min, Table II). The effect on MAO and also on SDH was more pronounced, value being 50% less than the basal value from 45-120 min (Table II). It was evident that the effect of muscimol on cholinesterases, lasted upto 45-60 min, while that on MAO and SDH, was much more pronounced and longer lasting (Table II).

When GABA antagonist picrotoxin was injected in III-V stimulation in the enzyme activities was observed. Highly significant increase in AChE was observed during 30-60 min (Table III). BuChE activity showed a steep rise and returned to the basal level at 120 min (Table III). After III-V injection of picrotoxin a statistical significant slow increase in MAO activity was observed at 30-60 min. This was true also for SDH enzyme activity (Table III).

TABLE II : Effect of third ventricle GABA agoinst muscimol on serum AChE, BuChE and MAO, and plasma SDH in male rats.

	AChE^{+}	*BuChE*$^{+}$	*MAO*$^{++}$	*SDH*$^{+++}$
Control	4.02±0 14 (100)	3.23±0.04 (100)	0.297±0.003 (100)	75.31±1.54 (100)
Post-injection				
1 min	3.72±0.70 (93)	2.80±0.04* (87)	0.282±0.003 (95)	69.14±1.54 (92)
15 min	3.08±0.05* (77)	2.34±0.04* (72)	0.234±0.003* (79)	59.07±2.16* (78)
30 min	2.43±0.04* (60)	1.76±0.03* (54)	0.213±0.007* (72)	39.72±3.05* (53)
45 min	2.13±0.05* (53)	2.41±0.03* (75)	0.142±0.005* (48)	32.28±2 43* (43)
60 min	2.25±0.05* (56)	2.77±0.02* (86)	0.143±0.003* (48)	32.27±1.47* (43)
120 min	3.72±0.04 (93)	3.57±0.05+ (110)	0.156±0.008* (53)	40.65±2.06* (54)

Values are Mean±SEM for n of 4-6 : parentheses, % of control.

Values are compared by one-way analysis of variance and then means are compared by Duncan's test (*P<0.05). +, units/mg protein, ++ μmoles of 4 - hydroxyquinoline/90 min/mg protein and +++ μg of TTC reduced/60 min/mg protein.

TABLE III : Effect of third ventricle GABA antagonist picrotoxin on serum AChE, BuChE and MAO, and plasma SDH in male rats.

	AChE^{+}	*BuChE*$^{+}$	*MAO*$^{++}$	*SDH*$^{+++}$
Control	4.05±0.06 (100)	3.08±0.03 (100)	0.309±0.007 (100)	70.83±1.84 (100)
Post-injection				
1 min	5.14±0.17* (127)	3.39±0.02* (1.0)	0.333±0.007 (108)	72.35±1.12 (102)
15 min	5.70±0.06* (141)	3.55±0.03* (115)	0.350±0.007 (113)	76.15±1.17* (107)
30 min	6.99±0.12* (173)	6.44±0.04* (209)	0.411±0.008* (133)	89.28±1.28* (126)
45 min	7.04±0.12* (174)	4 38±0.05* (142)	0.450±0.009* (146)	119.45±1.58* (169)
60 min	6.57±0.10* (162)	3.45±0.03* (112)	0.456±0.006* (147)	91.24±1.15* (129)
120 min	4.53±0.08* (112)	3.11±0.03* (101)	0.372±0.005* (120)	70.32±1.21 (99)

Values are Mean±SEM for n of 4-6 : parentheses, % of control.

Values are compared by one-way analysis of variance and then means are compared by Duncan's test (*P<0.05). +, units/mg protein, ++ μmoles of 4 - hydroxyquinoline/90 min/mg protein and +++, μg of TTC reduced/60 min/mg protein.

DISCUSSION

Our study has shown GABA and muscimol inhibited, while picrotoxin enhanced all these enzymes ie. AChE, BuChE, MAO and SDH. The inhibition can be explained as GABA and muscimol stimulate the release of norepinephrine (NE) [12,13]. NE raise c-AMP levels [14]. This c-AMP, in turn, raises ACh activity [15] and decreases AChE in CNS and blood [16]. Silman [17], also proposed that AChE and BuChE undergo parallel changes in normal and diseased conditions. In other words, this inhibition of AChE BuChE and possibly of MAO might be due to the inhibition in the NE acting through c-AMP. This indicates the interaction of GABA and muscimol with NE We have also seen earlier that the MAO enzyme changes occurring in hypothalamus are also reflected in blood [5].

Recently we have shown that centrally administered GABA and GABA selective agonist inhibit, while GABA antagonist enhances SDH enzyme activity in hypothalamic nuclei [4]. It is clear from our result that there is some sort of interaction between GABA ergic drugs and SDH in blood, but at this level we are unable to link them in a coordinated fashion.

It is clear that GABA and GABA selective agonist muscimol inhibit, while GABA antagonist picrotoxin stimulates the activity these enzymes in blood, involving two different neurochemical pathways.

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