

## EFFECT OF GABA, MUSCIMOL AND PICROTOXIN ON ELECTRICAL ACTIVITY OF THE MEDIAL PREOPTIC AREA IN UNANESTHETIZED RATS

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*Abstract.* Intracranial cannula along with electrodes was stereotaxically implanted in medial preoptic area of male rats. The electrical activity of mPOA was recorded before and after microinjection of GABA (0.5  $\mu\text{g}/0.2 \mu\text{l}$ ), muscimol (0.5  $\mu\text{g}/0.2 \mu\text{l}$ ), picrotoxin (0.25  $\mu\text{g}/0.2 \mu\text{l}$ ) and their respective controls. Generalized slowing with an increase in amplitude appeared with GABA and muscimol, while picrotoxin produced just the reverse, i.e. fast activity. Thus the mPOA seems to be vulnerable to GABAergic compounds and supports the diversity of physiological and behavioral functions.

Among diencephalic structures, medial preoptic area (mPOA) of the hypothalamus plays a prominent role in sleep (13) and EEG synchronization (17). Changes obtained in mPOA unit activity by brain stem reticular stimulation had been correlated with simultaneously induced variations in the cortical EEG (8). Certain experimental evidences also suggested the possible involvement of the midbrain reticular formation (MRF) in modulating the activity of mPOA (5, 9). In addition, there are anatomical studies showing projections of the MRF to the mPOA (11). Low frequency or single pulse stimulation delivered to the MRF has been shown to have either an excitatory (3) or an inhibitory influence (5) on mPOA. Since GABA is present in large amounts in the mPOA (18),

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investigations were designed to determine whether GABA, its agonist (muscimol) or antagonist (picrotoxin) injected directly into mPOA could alter the electrohypothalamogram recorded in unanaesthetized rats. Such a study in relation to mPOA has not so far been reported.

Twelve Wistar strain rats, weighing 200 to 250 g, were individually housed and maintained in a temperature ( $22 \pm 1^\circ\text{C}$ ) and light (14L : 10D) controlled room. For the experimental procedure, the animals were surgically prepared with intracranial cannula and recording electrodes under Nembutal (35 mg/kg b. wt., i.p.) anaesthesia. Bipolar stainless steel wire electrodes (10 mm length, 0.25 mm diameter, varnish insulated except for 0.5 mm at the tip) along with a cannula (17 mm length, 0.5 mm diam.) were placed using bregma as landmark (A 7.8, L 1.0, D 8.5) (4).

On the test day, individual rats were adapted to recording chamber. The rats were moved to a plastic tub which was placed in a shielded room. Electrical activity of mPOA was bipolarly recorded on a polygraph (Inco, India) at a time constant of 0.1 s and pass filter setting of 50 Hz. Following half an hour adaptation, all the three rats in each group were given intracranial injection (ICI) of GABA (0.5  $\mu\text{g}/0.2 \mu\text{l}$ ) or muscimol (0.5  $\mu\text{g}/0.2 \mu\text{l}$ ) or picrotoxin (0.25  $\mu\text{g}/0.2 \mu\text{l}$ ) along with their solvent (0.9% physiological saline, 0.2  $\mu\text{l}$ ). These substances were administered by slow microinjector at a speed of 0.1  $\mu\text{l}/\text{min}$ . In order to avoid ambiguity of results due to morphological changes at the site of injection, the injections were never repeated in any of these animals.

At the end of experimentation, rats were sacrificed under ether anaesthesia. Half  $\mu\text{l}$  of 2% ferric chloride was injected into the injection site using the injector cannula, brains were perfused with 3% potassium ferrocyanide in formol saline. Prussian blue reaction at the tips of cannula and electrodes in 25  $\mu\text{m}$  thick sections was utilized for identifying the sites of injection and recording electrodes.

The drugs used in the experiment were gamma-aminobutyric acid (Sigma, USA), muscimol (a research gift sample from Ciba-Geigy, Switzerland) and picrotoxin (Sisco Lab, India). All the drugs were administered intracranially into mPOA.

The data are presented as Mean  $\pm$  SEM (Table I). Statistical significance of differences between values is determined by analysis of variance (ANOVA) and Duncan's new multiple range test (10, 16).

Brains of 12 rats were explored for EEG activity from mPOA after intracranial injection (ICI) of GABA/muscimol/picrotoxin and their solvent (0.9% physiological saline). Basal range of pooled amplitudes and frequencies with ICI of GABA (20-85  $\mu\text{V}$ ; 3.9-5.2 cps), muscimol (20-65  $\mu\text{V}$ ; 3.9-4.6 cps), picrotoxin (15-55  $\mu\text{V}$ ; 4.8-6.1 cps) and their solvent (10-50  $\mu\text{V}$ ; 4.8-5.3 cps) was recorded from mPOA (Table I).

TABLE I

Correlation of means of frequencies ( $F$  in cps) and amplitudes ( $A$  in  $\mu V$ ) of total EEG record taken before and after ICI of saline, GABA, muscimol and picrotoxin in mPOA of the rat brain at various time intervals

Experimental manoeuvre		Minutes after injection						
		15	30	120	1	0	45	90
Saline	F	4.90 $\pm 0.02$	4.93 $\pm 0.04$	4.93 $\pm 0.12$	4.95 $\pm 0.06$	5.01 $\pm 0.06$	5.06 $\pm 0.10$	5.07 $\pm 0.09$
	A	26.50 $\pm 4.23$	26.50 $\pm 4.38$	27.00 $\pm 4.66$	27.00 $\pm 4.46$	27.00 $\pm 4.53$	27.50 $\pm 4.73$	28.00 $\pm 4.73$
GABA	F	3.45 $\pm 0.13$	3.58 $\pm 0.09$	3.62 $\pm 0.14$	3.72 $\pm 0.05$	3.75 $\pm 0.08$	4.25 $\pm 0.07$	4.29 $\pm 0.15$
	A	27.00 $\pm 2.63$	28.00 $\pm 2.96$	29.00 $\pm 3.41$	32.50 $\pm 5.17$	32.50 $\pm 4.53$	44.50 $\pm 7.87$	47.00 $\pm 8.32$
Muscimol	F	2.57 $\pm 0.06$	2.60 $\pm 0.05$	3.09 $\pm 0.07$	3.65 $\pm 0.14$	4.12 $\pm 0.08$	4.99 $\pm 0.39$	5.90 $\pm 0.30$
	A	20.00 $\pm 0.79$	22.50 $\pm 1.62$	23.00 $\pm 1.41$	33.00 $\pm 4.92$	40.00 $\pm 7.11$	41.00 $\pm 4.07$	147.50 $\pm 9.73$



Non-significant ( $P < 0.05$ ) results were observed after ICI of saline (Table I). The basal EEG record with saline remained almost unchanged throughout the recording period.

The GABA produced distinct changes in electrical activity of the brain. Within 15 min of its administration, slow and high amplitude (30-105  $\mu\text{V}$ ) waves superimposed with low voltage (20-60  $\mu\text{V}$ ) fast activity, began to appear. A generalized decrease in the frequency of basal activity was observed ( $P < 0.001$  and  $P < 0.01$  respectively). By the end of recording period, the voltage decreased (20-40  $\mu\text{V}$ ) and fast activity was recorded (Fig. 1, Table I).

Immediately after ICI of muscimol, a very conspicuous high voltage paroxysmal activity (110-180  $\mu\text{V}$ , 3.7-7.7 cps,  $P < 0.001$ ; at 15 min; Fig. 2)

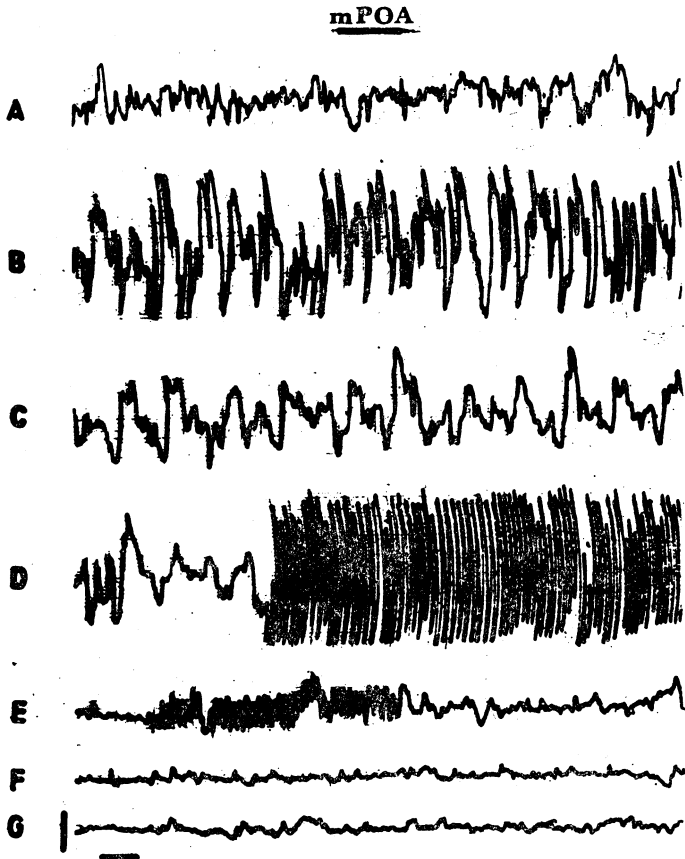


Fig. 2. Effect of application of muscimol on EEG recorded from rats. The upper tracing shows the ongoing activity before injection of the drug into mPOA (A) and recorded after 1 (B), 5 (C), 15 (D), 30 (E), 45 (F) and 120 (G) min of injection. Speed cal = 1 s. Amplification cal = 100  $\mu\text{V}$ .

became apparent from mPOA. This typical activity lasted almost for 5 min. At 15 min, high voltage slow waves superimposed with fast activity were recorded. However, later on, these activities remained a regular feature of the record, but every now and then, high voltage spiky seizures appeared. These, in their characteristics, looked exactly like epileptic discharges. Amplification, frequency and duration of occurrence of these discharges decreased with time (Fig. 2) and eventually disappeared (Fig. 2).

On the contrary, the response to ICI of picrotoxin was just the reverse to that of GABA and muscimol (Figs. 1-3). On the whole, the record, instead of becoming slow, became fast (4.8-6.1 cps to 5.8-8.1 cps at 15 min,  $P < 0.01$ ) and faster (5.8-8.1 cps to 5.8-9.4 cps at 30 min,  $P < 0.01$ ; Fig. 3). Later, instead of the fast activity, slower waves superimposed

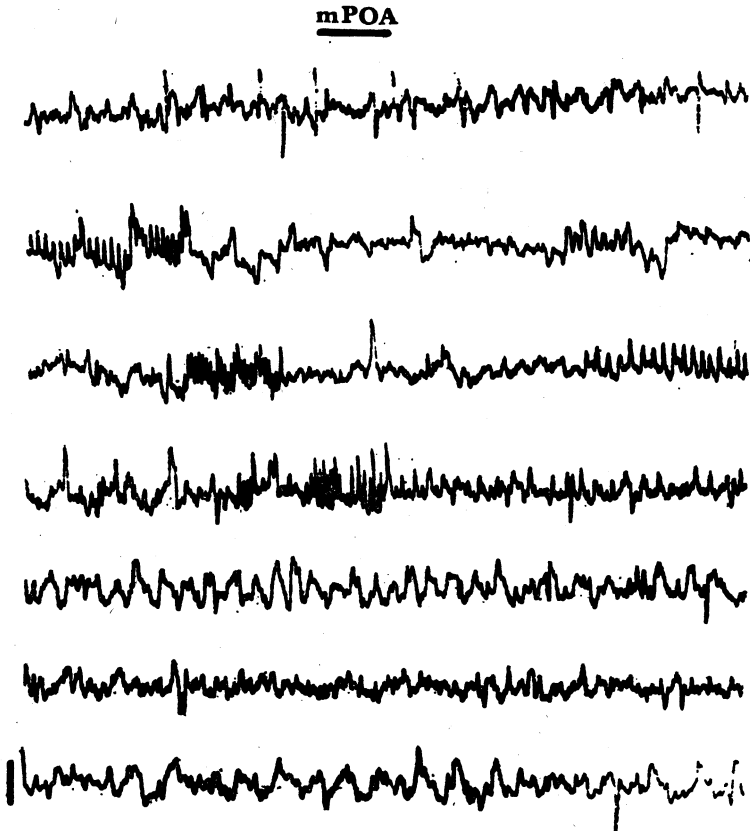


Fig. 3. Effect of application of picrotoxin on EEG recorded from rats. The upper tracing shows the ongoing activity before injection of the drug into mPOA (A) and recorded after 1 (B), 15 (C), 30 (D), 45 (E), 60 (F) and 120 (G) min of injection. Speed cal = 1 s. Amplification cal = 100  $\mu$ V.

with fast element appeared quite often and became a regular feature of the record.

The results have shown that an injection of GABA, muscimol and picrotoxin into mPOA caused synchronization of slow waves for a short period. The initial paroxysmal activity was changed into seizures and high voltage spikes with an apparent tendency towards seizure formation. This suggests that agonists of GABA probably had a tendency of charging the neuronal firing threshold of mPOA towards seizure formation, and picrotoxin just reversed this pattern to spikes of shorter duration, intermixed with low voltage slow waves. Similar findings have been reported in the medio-basal hypothalamic neurons (2, 9) where inhibitory agents like GABA, glycine and serotonin caused inhibition, while excitatory agents like glutamate, histamine and norepinephrine produced excitation of all neurons (2). Myslobodsky and Mansour (12) reported that gamma-vinyl-GABA induced EEG hypersynchronization in rats with prominent slow waves and paroxysmal spindle-like bursts of spike and wave activity. Behavioral and electrophysiological epileptiform phenomenon with neural GABAergic compounds like muscimol, imidazole acetate and sodium gamma hydroxybutyrate (6, 15) have also been reported, whereas the inhibitory mechanisms mediated by GABA have been presumed to be involved in controlling epileptic discharges (7).

The antagonism of muscimol and picrotoxin has also been shown to affect the concentration of succinic dehydrogenase (14). The interpretation of this antagonism can be proposed on the basis of two conformational sites of GABA receptors, one preferentially binding with agonist and the other with antagonist (1), differing not only in their affinity to various ligands but also in their locations and function. The concept thus derived, that electrical activity of mPOA is vulnerable to GABAergic compounds, probably supports the diversity of physiological and behavioral functions which it appears to manifest.

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