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GATING OF THE DORSAL PENILE NERVE INPUTS BY NOREPINEPHRINE AT THE MEDIAL PREOPTIC AREA IN RATS

H. N. MALLICK AND V. M. KUMAR*

Department of Physiology,
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi – 110 029

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Abstract : The medial preoptic area neurons related to male sexual behaviour in rats were identified by their responses to dorsal penile nerve stimulation. These neurons were further tested with norepinephrine applied iontophoretically. From the 21 medial preoptic area neurons recorded in urethane anaesthetized rats, 17 neurons responded to dorsal penile nerve stimulation. Excitatory and inhibitory responses were found in almost equal number of neurons. 14 neurons responded to norepinephrine application, out of which six neurons were excited and eight were inhibited. The direction of changes produced by dorsal penile nerve stimulation and norepinephrine application were similar in 10 neurons. The results suggest that the sensory inputs from the genitalia are possibly gated by norepinephrine at the level of the medial preoptic area. Afferent information from the genitalia carried by dorsal penile nerve and the availability of norepinephrine at the level of the medial preoptic area probably help in maintaining adequate level of sexual arousal.

Key words : medial preoptic area norepinephrine dorsal penile nerve microiontophoreois sexual behaviour

INTRODUCTION

The medial preoptic area (mPOA) is a key neural site in the regulation of male sexual behaviour (1, 2). The mPOA neurons show changes in firing rates during different phases of male sexual behaviour in rats and monkeys (3, 4). These studies have shown the involvement of the mPOA in both sexual arousal and copulatory performance. Stimulation of the dorsal penile nerve (DPN) which carries sensory information from the genitalia, influences the mPOA neuronal activity (5). Some of the dorsal penile nerve afferents synapse at various nuclear groups in the brainstem, before projecting to the mPOA. The mPOA receives dense inputs from the brainstem noradrenergic cell groups (6). Injection of norepinephrine (NE) into the mPOA facilitates most of the components of the male sexual behaviour and also increases wakefulness (7, 8). NE applied directly either excited or inhibited the mPOA neurons in the anaesthetised animals (9). The present study was undertaken to find out the responses of the sex related mPOA neurons to iontophoretic application of NE in anaesthetised rats. The

*Corresponding Author : Email : v_mohan_kumar@hotmail.com; Fax : 91-11-26588663

sex related neurons were identified by the responses of the mPOA neurons to DPN stimulation.

MATERIALS AND METHODS

Experiments were carried out on 10 male Wistar rats anesthetised with urethane (1.5 g/kg, BW, IP). Body temperature was maintained in between 37.0 and 37.5°C by a hot water pad. The head of the rat was fixed to the stereotaxic apparatus. The skull was exposed and 2 mm diameter hole was drilled bilaterally on the skull area above the mPOA for recording single unit activity. The DPN was exposed on the dorsum of the penis and was isolated from the surrounding tissues for stimulation.

Piggyback multibarrel microelectrode assembly was used for single unit recording and for iontophoretic application of drugs. Three-barrel glass capillaries (Kwik-Fil glass capillaries, WPI, USA) were pulled using a vertical electrode puller (Narishige Scientific Instrument Laboratories, Japan) and broken to a tip diameter of 4–5 µm. Single barrel glass capillaries were (1.5 mm o.d.) pulled to tip diameter of 1 µm for the recording electrodes. The recording electrode was glued to the mutibarrel microelectode at the site with dental cement so that its tip was protruding 15–20 µm beyond the ejecting barrels. The recording electrode was filled with 3M sodium acetate mixed with 2% pontamine sky blue. The iontophoretic barrels were filled with L-glutamate (2M), noradrenaline bitartrate (0.5 M) and NaCl (0.5 M) solutions. The drug solutions were filtered with 0.2 µm microfilter. The resistance of the recording electrode was kept in between 1 and 5 MΩ, and that of the drug containing barrels were kept in between 70 and 90 MΩ. A retaining current of 15–20 nA was applied to prevent drug diffusion. Extracellular action potentials were recorded from the mPOA, as per methods described earlier (5). The electrode assembly was lowered to the mPOA through the hole made, using a hydraulic microdrive (Model MO-81, Narishige Scientific Instrument Laboratories, Japan) as per coordinates from the DeGroot stereotaxic atlas (10). Once a stable unit was obtained, the DPN was stimulated as per methods described earlier (5). After DPN stimulation, the mPOA neurons were subjected to iontophoretic application of NE. Drugs were applied from a WPI model M-707 microprobe system. The drugs were ejected using 30–90 nA current of appropriate polarity for a duration of 10–20 s. Glutamate was applied to determine if the multibarrel electrode assembly was intact with the recording electrode and at a distance close enough for the drugs to exert their effect. Specific chemical effect was distinguished from the current effect based on the criteria described by Stone (10). At the end of the recording sessions, the electrode sites were marked by ejecting pontamine sky blue through the recording electrode by passing 10 A current for 15 min. The brains were fixed with 150 ml of 30% formaldehyde by intracardiac perfusion and then removed and preserved in 10% formaldehyde solution. 10 µm paraffin fixed sections were processed for histological verification of the recording sites.

RESULTS

Extracellular action potentials were recorded from 21 mPOA neurons. The data include those neurons, which responded to glutamate but not to NaCl. A typical response of an mPOA neuron to iontophoretic protocol used is shown in Fig. 1. Responses of a neuron to six repeated stimulations of the DPN and three iontophoretic applications of NE were averaged. The data were analysed in epochs of 10 s each. Firing rates for 10 s each during the pre stimulation, stimulation and post stimulation periods were analysed using Normal test. The response of the unit was considered significant if the z value was more than 1.6.

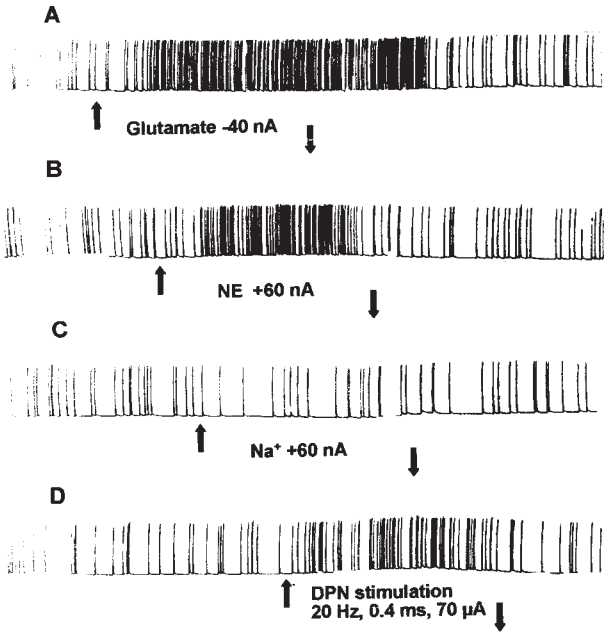

Fig. 1 : Polygraphic tracings showing responses of a medial preoptic area neuron to iontophoretic applications of glutamate (A), norepinephrine (NE) (B), Na^+ (C) and stimulation of dorsal penile nerve (D). Beginning and end of current applications are shown as arrows.

Out of the 21 neurons recorded, 17 neurons responded to DPN stimulation, whereas 14 neurons responded to NE application (Table I). The baseline firing rate of mPOA neurons obtained from 21 neurons were in between 0.5 to 35.8 Hz. (Mean ± SD, 7.62 ± 10.21). DPN stimulation in 21 neurons produced inhibition in 8 and excitation in 9. Iontophoretic applications of NE in the vicinity of these neurons produced inhibition in 8 and excitation in 6. The direction of changes by DPN stimulation and iontophoretic NE application were similar in 10 neurons. Out of these, five neurons, which were excited by DPN stimulation, were also excited by NE application. Another five neurons, which were inhibited by DPN stimulation, were also inhibited by iontophoretic application of NE. Reversal of excitatory response by DPN stimulation to inhibition by NE application was observed in three neurons. One neuron responded to NE but not to DPN

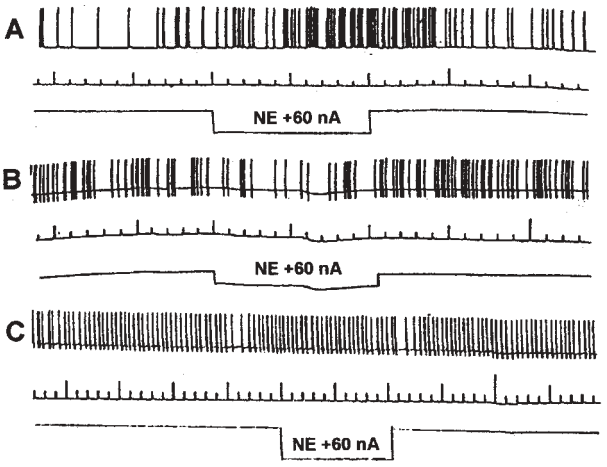

Fig. 2 : Effect of iontophoretic applications of norepinephrine (NE) on three different medial preoptic area neurons. Neuron A shows increase whereas neuron B shows decrease in firing rate. Neuron C did not respond.

TABLE I: The mean firing rate of 21 preoptic area neurons (mPOA) neurons (Mean±SD) before, during and after dorsal penile nerve stimulation and iontophoretic application of noreinephrine (NE).

No. of Neurons	*DPN stimulation*			*NE application*		
	Before	*During*	*After*	*Before*	*During*	*After*
1.	4.53±1.0	3.42±0.45*	3.93±0.43	5.06±0.76	4.2±0.26*	5.06±0.64
2.	5.2±0.14	5.43±0.3	5.23±0.23	5.33±0.76	5.96±1.0	5.46±0.46
3.	38.5±4.04	28.5±9.11*	39.0±3.82	25.0±3.69	23.7±4.57	22.25±1.7
4.	4.1±0.53	8.15±0.63*	5.1±0.8	7.16±1.3	9.5±0.97*	12.92±1.7*
5.	7.14±0.74	5.98±0.49*	5.96±0.65*	6.68±0.52	5.8±0.66*	5.82±0.94*
6.	5.21±0.14	6.53±0.72*	6.2±0.37	4.93±0.11	2.43±0.37*	3.76±1.53
7.	1.78±0.33	3.58±0.41*	1.91±0.41	1.90±0.1	1.23±0.03*	1.66±0.05
8.	3.60±1.27	5.03±1.49*	3.03±1.00	4.75±0.07	3.25±0.67*	2.7±0.14*
9.	6.73±0.90	6.3±0.79	6.33±0.05	2.47±0.53	3.05±0.68	2.55±0.51
10.	1.2±0.0	0.7±0.1*	1.1±0.09	1.13±0.2	0.33±0.11*	1.11±0.2
11.	4.6±1.21	14.26±3.1*	4.06±0.11	6.45±0.23	6.10±0.11	6.47±0.44
12.	1.53±0.11	3.6±0.26*	1.46±0.15	1.06±0.23	1.76±0.05*	1.40±0.54*
13.	11.7±1.97	13.6±2.88	11.2±1.15	8.92±1.58	9.84±2.16	9.32±1.30
14.	9.89±2.10	16.3±3.39*	9.13±2.24	7.65±0.52	9.17±0.62*	13.0±0.34*
15.	4.72±0.99	3.70±0.64*	4.40±1.00	5.50±0.62	4.4±0.38	4.90±0.60
16.	4.85±0.76	4.75±1.38	5.02±0.86	4.90±0.21	6.25±0.92*	5.82±0.41*
17.	35.8±6.22	24.06±2.5*	37.3±6.11	22.5±5.54	20.5±5.07	20.9±4.43
18.	0.51±0.2	1.91±0.17*	1.43±0.18	0.46±0.15	1.50±0.20*	1.06±0.11*
19.	5.23±0.23	6.6±0.42*	5.86±0.35	3.66±0.73	5.86±0.32*	4.96±1.04*
20.	1.72±0.55	0.58±0.19*	1.4±0.55	1.9±0.74	0.81±–.35*	1.80±0.48
21.	1.5±0.26	0.46±0.15*	1.53±0.05	1.02±0.29	0.90±0.27	1.0±0.33

The significant changes (*) indicate Z value more than 1.96 obtained after applying normal test.

stimulation. Three neurons which responded to DPN stimulation were not affected by NE application. The excitatory and inhibitory responses of two mPOA neurons to iontophoretic NE application are shown in Fig 2. The effect of NE lasted for 20 *s* 1.6 min.

DISCUSSION

The present study is the first report on the effect of NE on the mPOA neurons responsive to genital afferent stimulation. The effects of the DPN stimulation on the mPOA neuronal activity have been discussed in detail in our previous report (5). In the present study, the DPN was stimulated to identify the neurons in the mPOA responsive to genital afferent inputs. These neurons are designated as sex related neurons in this study. These sex related neurons were further subjected to iontophoretic application of NE, which produced both excitation as well as inhibition.

NE application produced both inhibitory as well as excitatory responses on the mPOA neurons. Local application of NE by iontophoresis suppressed neuronal discharge in the cerebral cortex, cerebellum, hippocampus, thalamus, hypothalamus and preoptic area (12–18). These depressant actions are also mimicked and blocked by agonists and antagonists respectively. These findings support the notion that NE is primarily an inhibitory transmitter at central synapses including the POA. But in our study a significant population of the mPOA neurons showed excitatory responses to iontophoretic NE application besides inhibitory responses. NE also has been shown to produce excitatory responses in paraventricular nucleus (18). One plausible explanation for the dual response of sex related neurons to NE could be that it is mediated by different noradrenergic receptors, although this issue was not addressed in this study. Excitatory response to noradrenaline has been shown to be mediated by α_1 receptor whereas inhibitory response has been shown to be mediated by β receptor (18). There is change in firing rates of the mPOA neurons during different phases of male copulatory behaviour (3, 4). In interpreting the responses of iontophoretic NE application on the sex related neurons of the mPOA, it is also necessary to explain them in relation to behavioural context. The results of this study indicate that NE is capable of increasing as well as decreasing the mean firing rate of the mPOA neurons. Therefore, in a behavioural situation like copulation where there is moment to moment change in firing rate of the mPOA neurons, NE probably has a modulatory role.

It is also important to consider at this stage, the ability of NE to influence spontaneous firing rate of mPOA neurons. However such ability does not necessarily explain its contribution to the mPOA neuronal circuit, which is involved in such a complex behaviour like copulation. The afferent inputs from DPN to the mPOA produce both excitatory and inhibitory responses. The dual actions of NE in the mPOA may be reinforcing these phasic inputs from the glans penis. Results also suggest the possibility of excitatory input being changed to an inhibitory one by endogenously available NE. Such examples of gating action of NE have been shown in certain behavioural situations (19, 20). Therefore, it is suggested that the influence of phasic excitatory or inhibitory inputs from the DPN on the mPOA neuronal firing can be modulated by local level of NE, dictating the gain of a specific input, which may be requiring amplification for maintenance of a given behavioural state. Afferent information from the genitalia (carried by the DPN) and the release of NE at the level of the mPOA, probably help in maintaining adequate level of sexual arousal.

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