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Effects of NMDA and non-NMDA ionotropic glutamate receptors in the medial preoptic area on body temperature in awake rats ☆

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ABSTRACT

Glutamate when microinjected at the medial preoptic area (mPOA) influences brain temperature (T_{br}) and body temperature (T_b) in rats. Glutamate and its various receptors are present at the mPOA. The aim of this study was to identify the contribution of each of the ionotropic glutamatergic receptors at the mPOA on changes in T_{br} and T_b in freely moving rats. Adult male Wistar rats (n=40) were implanted with bilateral guide cannula with indwelling styli above the mPOA. A telemetric transmitter was implanted at the peritoneum to record T_b and locomotor activity (LMA). A precalibrated thermocouple wire implanted near the hypothalamus was used to assess T_{br}. Specific agonist for each ionotropic glutamate receptor was microinjected into the mPOA and its effects on temperature and LMA were measured in the rats. The rats were also microinjected with the respective ionotropic receptor antagonists, 15 min prior to the microinjection of each agonist. Amongst amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) and kainic acid, AMPA increased T_b and LMA when injected at the mPOA. Specific antagonists for AMPA receptors was able to attenuate this increase ($p < 0.005$). Pharmacological blockade of NMDA was able to lower T_{br} only. Microinjection of kainic acid and its antagonist had no effect on the variables. The finding of the study suggests that activation of the AMPA receptors at the mPOA, leads to the rise in body temperature.

1. Introduction

The preoptic area (POA) plays a crucial role in thermoregulation. It receives thermal information from the periphery and the brain and initiates appropriate thermoregulatory responses (Hammel, 1965; Hammel et al., 1963). The thermoregulatory responses are exercised through neural connections between hypothalamus and other caudal thermo-effector areas (Nagashima et al., 2000). Experiments done on various species have shown the influence of many endogenous substances and neurotransmitters on thermoregulation when injected at the mPOA. Microinjections of serotonin, acetylcholine, norepinephrine, histamine, growth hormone releasing hormone (GHRH), ɣ-amino butyric acid (GABA), glutamate and prostaglandin E2 (PGE_2) into the different regions of the POA, mainly lateral and medial parts affects temperature in rats and other non-human primates (Crawshaw, 1972; Gatti and Gertner, 1984; Mallick et al., 1988; Osaka, 2004; Poole and Stephenson, 1979; Sengupta et al., 2014; Talwar and Kumar, 1994; Zaretsky et al., 2006; Zhang et al., 1999).

L-glutamate is the major excitatory neurotransmitter in the central nervous system. Rat hypothalamus has receptors for both metabotropic as well ionotropic glutamatergic receptors (Eyigor et al., 2001; van den Pol et al., 1994; van den Pol and Trombley, 1993). L-glutamate injection into the POA suppresses shivering in cold-exposed anaesthetised rats (Zhang et al., 1995). The injection of ionotropic glutamate agonist, N-methyl D-aspartate (NMDA) into the median preoptic nucleus (MnPO) of anaesthetised rats increases T_b, but has no effect when injected into the mPOA and the lateral preoptic area (lPOA)(Nakamura and Morrison, 2008). In contrast, microinjection of the inhibitory amino acid, GABA into the POA elicits thermogenic and hyperthermic responses in anaesthetised rats (Osaka, 2012). In our previous study it was reported that glutamate microinjection in the POA in unanesthetised rats increased T_b and T_{br} (Sengupta et al., 2014). In the present study, we examined the role of each ionotropic glutamatergic receptors at the mPOA on T_b, T_{br} and LMA in awake behaving rats.

2. Material and methods

2.1. Animals and housing

The study was carried out on 40 adult male Wistar rats weighing 200–250 g, procured from the Central Animal Facility of

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All India Institute of Medical Sciences (New Delhi, India). The rats were housed in individual transparent polysulphone cages and given *ad libitum* access to rat chow and water. They were exposed to a 14:10 light: dark cycle with light-on period from 6:00 to 20:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee and was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.2. Drugs and vehicle for microinjection

The drugs used in this study included NMDA, NMDA receptor antagonist, D (-)-2-amino-5-phosphonopentanoic acid (AP5), AMPA, AMPA receptor antagonist, D-Ɣ-glutamyl amino methane sulphonic acid (GAMS), kainic acid and kainic acid receptor antagonist, 5-nitro-6,7,8,9-tetrahydrobenzo [g]indole-2,3-dione-3-oxime (NS102). All the drugs were purchased from Sigma-Aldrich (St Louis, Missouri, USA). These drugs were dissolved in 0.9% pyrogen-free saline, and solutions were adjusted to pH 7.0. The 0.9% saline was also used as the control vehicle microinjection. Control saline and drug solutions were freshly prepared under sterile conditions before each use.

2.3. Surgical procedures

Under sodium pentobarbitone anaesthesia (40 mg/kg BW, IP), the rats were fixed to the stereotaxic apparatus (Narishige Scientific Instruments laboratories, Tokyo, Japan). A bilateral guide cannula made of 23 G stainless steel tube with a 30 G indwelling styli of equal length was chronically implanted 2 mm above the mPOA (A 7.8, V 7.5, and L 0.6) according to DeGroot's atlas (DeGroot, 1959). For the assessment of T_{br} from near the POA, a precalibrated K-type thermocouple wire was lowered along the midline, between the duramater and piamater at an angle of 25° anteroposteriorly to a height of 4.5 mm (A 9.0, according to DeGroot's atlas) as mentioned in the previous work (Srividya et al., 2006). To record T_b and LMA, a transmitter capable of sensing temperature and activity (TA10TAF40, 7.15 g and 3 × 1.3 × 0.8 cm; (Dataquest ART; Data Sciences International, St Paul, Minnesota, USA)) was surgically implanted into the rats' peritoneum. The rats were given 7–10 days of postoperative recovery and then habituated for 24 h in the recording chamber, maintained at an ambient temperature of 25 ± 1.1 °C. T_{br} was recorded in the freely moving rats with a digital thermometer (Fluke Multimeter; Fluke, Everett, Washington, USA) connected to the K-type thermocouple. T_b and LMA were recorded by telemetry (Dataquest ART; Data Sciences International, St Paul, Minnesota, USA).

2.4. Experimental design and microinjection of drugs at the mPOA

The effects of microinjection of ionotropic glutamate receptor agonists and antagonists (NMDA, AP5+NMDA, AMPA, GAMS+AMPA, kainic acid and NS102+kainic acid) were studied on T_b, T_{br} and LMA in seven groups of rats. T_b, T_{br}, and LMA were recorded from 10:00 to16:00 h.

On the day of microinjection, 20 ng of NMDA (Sigma- Aldrich, St Louis, Missouri, USA) dissolved in 200 nl of normal saline (0.14 nM) was microinjected into the mPOA at 12:00 h, using 30-G injector cannula, introduced through the guide cannula, in one group (n=5). This dose is in equimolar concentration of the dose of L-glutamate as used in the previous study; based on the pilot work conducted on four rats with 10, 20, 40, and 80 ng of L-glutamate in 200 nl saline (0.07, 0.14, 0.27, and 0.5 nM respectively). The lowest dose (0.14 nM L-glutamate) which produced an appreciable change (> 0.5 °C in T_b) was selected for further study (Sengupta et al., 2014). To confirm the receptor specificity of the effect of NMDA, in the second group of rats, equimolar (0.14 nM) ionotropic receptor antagonist, AP5 (Sigma-Aldrich, St Louis, Missouri, USA) dissolved in 200 nl of normal saline was microinjected into the mPOA at 11:45 h. This was followed by a second injection of NMDA (0.14 nM) at the same site, using the same injector cannula, after 15 min. Similar dose and recording schedule was followed for AMPA and its antagonist GAMS. Equimolar dose of kainic acid microinjection as that of the other two excitatory amino acids in the behaving rats caused seizure-like activity, circling behaviour and ultimately cell body lesion (Sperk et al., 1985). So we used a lower dose for kainic acid (0.06 nM) (Silveira and Graeff, 1992) in the other groups (n=5). Again to confirm the receptor specificity of AMPA and kainic acid, two other groups (n=10) were microinjected with 200 nl of equimolar solution of AMPA antagonist, GAMS and kainic acid antagonist, NS102 into the mPOA at 11:45 h. This was followed by microinjection of AMPA and kainic acid at the same site using the same injector cannula. Pyrogen-free physiological saline (0.9%, volume 200 nl), used as a vehicle, was injected in the control group (n=6) at 12:00 h.

2.5. Histological localisation of the microinjection sites

At the end of the experiment, each rat was deeply anaesthetised with an overdose of sodium pentobarbitone (50 mg/kg BW, IP). The injection sites were marked by injecting 200 nl of 2% ferric chloride through the same injector cannula, with which microinjection was performed. The brains were fixed with 10% formalin given intracardially. The brains were then dissected out for histology. Ten micrometre brain sections were stained with hematoxylin and eosin, to identify the brain area and provide a contrast to the site of injection, visible as Prussian blue dot (Fig. 1A and B).

2.6. Data analysis

The temperature was recorded continuously at an interval of 15 s for 6 h, and the data were averaged for 15 min segments (mean ± SD) for statistical analysis. Every 1 h post-injection effect of the drug on T_b, T_{br} and LMA was compared to the average of 2 h pre-injection using one-way repeated measures of ANOVA followed by Bonferroni post-hoc test. A Mann-Whitney *U* test was performed to see the significant difference in T_b, T_{br} and LMA between agonist and antagonist+agonist groups. For all comparison, statistical significance was set at $p < 0.05$. Data was analysed using IBM SPSS version 20 (New York, USA).

3. Results

A total of 108 sets of microinjections were made in 40 mPOA injection sites. The results from 36 rats in which the injection sites were confirmed within the mPOA are described here (Fig. 1A and B). The 2 h average pre-injection recordings of T_b and T_{br} for AMPA injected group was 37.8 ± 0.1 and 37.6 ± 0.1 °C, for the GAMS+AMPA injected group was 37.1 ± 0.11 and 38.1 ± 0.14 °C, whereas, for NMDA injected group was 37.5 ± 0.1 and 37.6 ± 0.07 °C, for the AP5+NMDA injected group was 37.7 ± 0.1 and 37.7 ± 0.2 °C respectively (Fig. 2A and B). For the kainic acid injected group, the 2 h pre-injection data was 37.5 ± 0.2 and 37.5 ± 0.1 °C, for the NS102+kainic acid injected group, it was 37.7 ± 0.1 and 37.6 ± 0.08 °C, respectively (Fig. 2C).

AMPA microinjection in the mPOA increased T_b significantly, ranging from 38.4 ± 0.9 °C to 38.9 ± 1.2 °C ($F_{1,\ 5}=3.89$, $p < 0.005$, $F_{1,\ 5}=3.75$, $p < 0.05$) when compared to the 2 h average of pre-injection data (Fig. 2A). GAMS when injected 15 min prior to AMPA was able to block the rise in both T_b (U=15.6, p=0.038, U=20.23,