Indian J Physiol Pharmacol 2004; 48 (4) : 471–475

REPEATED INTRACEREBRAL MICROINJECTIONS : EFFICACY IN STUDYING BRAIN FUNCTIONS

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(Received on May 26, 2004)

Abstract : Injection of chemicals into the brain has been considered as an important technique to study various functions of the brain. In these studies, as a rule, only one bilateral injection is given in one animal. This study was undertaken to evaluate the quality of the body temperature data obtained after first and second injections of methoxamine and artificial cerebrospinal fluid into the medial preoptic area.

Though there was quantitative decrease in the effects produced after the second injection of the drug, there was no significant change in the effects produced by the second injections of artificial cerebrospinal fluid, which was used as a vehicle.

Results of this study support the earlier recommendation to perform only one injection in any of the brain sites for evaluating the effect of any drug. But the vehicle can be administered as a second injection, without compromising on the quality of data.

Key words : artificial cerebrospinal fluid methods microinjection hypothermia methoxamine body temperature multiple injections preoptic area rats telemetry

INTRODUCTION

Microinjection of chemical substances into the specific areas of the brain in free moving animals has been used in neuro science research for at least 50 years to explore various functions of the brain areas and specific neurotransmitter systems (1, 2). The technical details of the construction of the cannulae and the injection procedure have been reviewed (3, 4). These guidelines recommend penetration/injection into the brain area in an animal may be restricted to just once, as morphological and neurochemical changes were produced at the injection site (4). On the contrary, no evidence of tissue damage was reported even in rats that had received multiple injections in the hypothalamus with the exception of guide path (5). No significant difference in drinking was detected between the first and second injections of angiotensin-II into

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the lateral preoptic area (6). Moreover, the relative unavailability of the animals and ethical considerations demand reduction in the usage of animals in experimental procedures. So, in this study, the quality of data from second microinjection at the same site of the brain was evaluated.

The role of the preoptic area in the regulation of body temperature (T_b) is well documented, and α_1-adrenoceptors in the mPOA have been implicated in bringing about a fall in T_b (7, 8, 9, 10, 11). Here, in this study, we compare the effect of an α_1 agonist methoxamine and vehicle on T_b, when microinjected into the medial preoptic area (mPOA) either as first injection or second injection.

METHODS

The study was conducted on ten male adult Wistar rats weighing 200–230 gms obtained from Experimental Animal Facility of All India Institute of Medical Sciences, New Delhi, India. The animals were kept in the animal room for at least four weeks prior to the surgical procedure with ad libitum access to food and water and 14 hrs/ 10 hrs light/dark cycle (lights on at 06:00 hrs). The animal house temperature was maintained at 26±1°C.

Under pentobarbitone sodium (Aldrich Thomas Co, USA) anaesthesia (40 mg/kg body weight, i.p.), rats were chronically implanted with a bilateral guide cannulae aimed at 2 mm above the mPOA (12, 13), for the injection of drugs, as per De Groot atlas (14). They were also implanted with a radio transmitter (Data Sciences International, USA) for the recording of T_b (15).

Experiments were carried out in a sound attenuated chamber maintained at 26±1°C temperature. The animals were introduced into the chamber with the transmitter switched on, at least one day before the experiment and left undisturbed. On the recording day, continuous telemetric recording of intraperitoneal temperature (DATA QUEST 1.1®, Data Sciences International, USA) was done for 2 hrs before and 3 hrs after the intracerebral injection. The recording started at 10:00 hrs and was terminated at 15:10 hrs, though it was discontinued during the period of injection (i.e. from 12:00 to 12:10 hrs).

The animals were divided into 2 groups. The data of 5 animals of each group, which received injection in the mPOA was only included in the results. One group of animals received the vehicle, artificial cerebrospinal fluid (aCSF) and the second group received 1 µmol methoxamine dissolved in aCSF as first injection. After 7 days, first group of animals received 1 µmol methoxamine and the second group received aCSF. By using Limulus amoebocyte lysate (LAL) test, the aCSF was tested for pyrogens (16). Injections were given bilaterally (0.2 µl) at a rate of 0.1 µl/min using an injector cannula made up of 30 G stainless steel tubing. All the injections were performed at the same time (12:00 hrs) to avoid the effect of circadian variation in the results.

Preinjection data obtained from each group was analysed by the non-parametric

two-way analysis of variance (Friedman's test). Preinjection values of aCSF injections were compared with their own postinjection values using Friedman's multiple range test. The postinjection values of first and second injections of methoxamine were compared with first and second injections of aCSF using the Mann Whitney test.

At the end of the experiment, the brains of the animals were perfused with formaldehyde-saline as described earlier for histological confirmation of drug injection site (17).

RESULTS

The preinjection data of T_b obtained from different animals did not show any significant difference. Microinjection of pyrogen free aCSF into the mPOA, either as first or second injection, caused a gradual increase in T_b when compared with their respective preinjection controls (Fig. 1). The increases in T_b by the end of the 3 hrs after first and second injections (1.52±0.55°C and 1.39±0.76°C respectively) were comparable. Changes of individual time bin values of T_b, after first and second injections, were nearly identical (Fig. 1).

1 µmol of methoxamine caused a fall in T_b by 1.28°C, followed by a rebound increase, after first injection. The fall in T_b had a nadir around 45–60 min. On the other hand, second injection produced lesser fall in T_b (0.37°C) and its nadir was at 30–45 min after the injection. The differences were apparent on readings obtained at different time bins (Fig. 1).

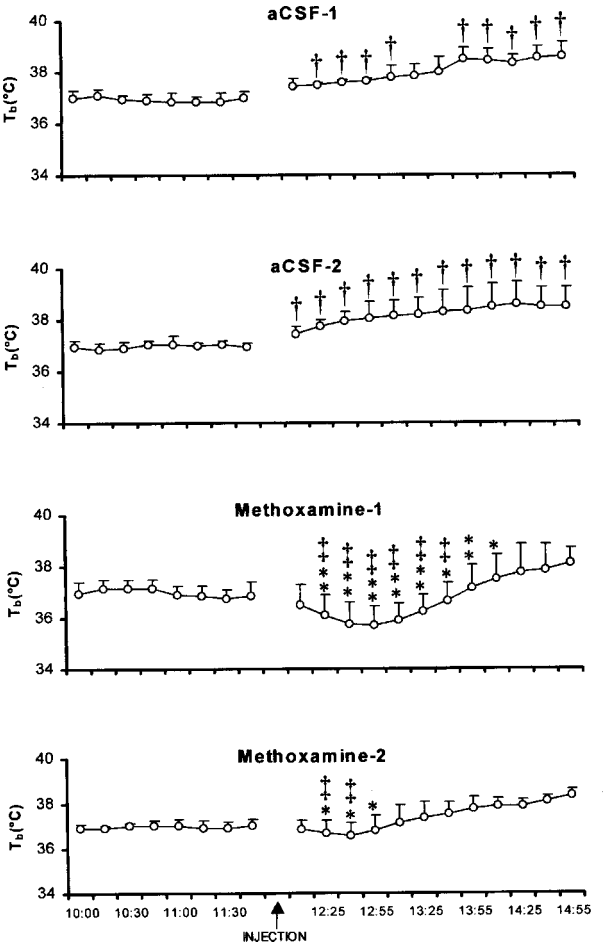

Fig. 1: Body temperatures after the injection of aCSF/methoxamine (1 µmol) at the medial preoptic area either as first or second injections. Point of recording, at which drug was injected is shown by the arrow. Data are mean±S.D.

**P<0.01, *P<0.05 significance compared to the aCSF-1 values.

‡P<0.01 significance compared aCSF-2 values. †P<0.05 significance compared to their respective preinjection values.

aCSF-1 – injection of aCSF as first injection; aCSF-2 – injection of aCSF as second injection; Methoxamine-1 – injection of methoxamine as first injection;

Methoxamine-2 – injection of methoxamine as second injection.

DISCUSSION

Microinjection of aCSF into the mPOA, either as a first injection or as a second injection, produced the same magnitude of increase in T_b. Magnitude of fall in T_b after the second methoxamine injection was less than the first.

The hyperthermia observed after the injection of aCSF into the mPOA could be due to acute tissue injury and release of prostaglandins and cytokines as cytokines and prostaglandins are released in response to brain injuries (18, 19, 20). These chemicals are reported to cause changes in T_b, when applied into the mPOA (19, 21).

Methoxamine injection into the mPOA produced an initial fall in T_b. This is in line with the earlier reports and further supports the idea that the α_1 adrenergic receptors might be mediating this change (8, 9). The fall in T_b after injection of adrenergic agonist was suggested to be due either to a non-evaporative heat loss or to a decrease in heat producing mechanisms (22, 23). Methoxamine microdialyzed into the mPOA did not cause any change in skin temperature (24). So, the hypothermia induced by methoxamine might be due to a decrease in heat production.

The change induced by acute tissue injury (i.e. aCSF induced increase in T_b) is reproduced, almost without decrement, in the second injection. On the other hand, the drug (i.e. Methoxamine) induced changes are reduced in the second injection. Our results support the earlier suggestion that only one injection be given in any of the brain sites (4). But the results suggest that microinjection of control materials, for eg. aCSF, can be performed without compromising on the quality of the data.

Scientific journals and ethical committees stress on minimising the number of animals in any of the experimental protocol (http://authors.elsevier.com, http://ftp.grants.nih.gov/IACUC/GuideBook.pdf. http://www.nap.edu/readingroom/books/labrats/chaps.html, http://www.homeoffice.go.uk/docs2/regtoxicologydraftrevision4_03.html). All the microinjection studies require a group of animals, in which only the control material is injected. This would involve use of additional number of animals. If the vehicle is injected in the same animals used for drug injection, after a small recovery period, it will be helpful in minimizing the number of animals. In addition, it would minimize the workload and time involved in the study. This would also help to reduce the animal variability since the drug and vehicle injections are performed in the same animals.

Based on the present findings, it is suggested that the vehicle of the drugs can be administered as second intracerebral injection, without compromising on the quality of data.

ACKNOWLEDGEMENTS

This study was supported by Council of Scientific and Industrial Research, India.

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