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A POSSIBLE ROLE OF THE SEROTONERGIC SYSTEM IN THERMOREGULATION IN THE RABBIT

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Summary—In order to examine the possible role of the serotonergic system in the brain stem on thermoregulation, lysergic acid diethylamide (LSD) was administered intravenously or locally into the nucleus raphe dorsalis (NRD) or the nucleus raphe magnus (NRM) of rabbits at various ambient temperatures. Intravenously administered LSD (12–17 μg/kg) caused hyperthermic responses in the ambient temperatures of 15, 25 and 35°C. The magnitude of these responses was greater between 15 and 25°C than it was at 35°C. Injected locally, LSD also caused an increase in the rectal temperature and a fall in the ear skin temperature. These changes were dependent on the dose of LSD administered in both the NRD and the NRM groups. With local administration of LSD into the NRD, heat production increased slightly even at the ambient temperature of 29°C. On the other hand, no changes occured over the 180 min period following administration of the drug into the NRM. From these results it could be concluded that the afferent input through cold fibers makes a synapse with 5-HT inhibitory receptors on the warm-responsive neurons in the midbrain and also connects with an excitatory synapse in the heat production system. It could also be concluded that LSD acts on these receptors as an agonist or an antagonist, consequently inducing a hyperthermic reaction.

The hypothesis has been proposed by Feldberg and Myers (1964) that body temperature in the homeotherm is controlled by balance of opposing actions due to the relative rates of catecholamine and 5-hydroxytryptamine (5-HT) released in the hypothalamus; 5-HT, especially, has been understood to be a mediator in thermoregulatory heat loss system (Hellon, 1975). Considerable evidence supports the idea that peripheral vasodilatation is evoked by microinjection of 5-HT into the cerebral ventricles of rats or rabbits (Banerjee, Burks, Feldberg and Goodrich, 1970; Crawshaw, 1972). Systemic or intraventricular injections of 5-hydroxytryptophan (5-HTP), a 5-HT precursor, produced a fall (Jacob, Girault and Peindaries, 1972; Tangri, Bhargava and Bhargava, 1974) or a rise (Horita and Gogerty, 1958; Horita and Hill, 1973) in the body temperature of rabbits. On the other hand, it has also been generally believed that the activity of temperature-responsive neurons in the preoptic/anterior hypothalamic (PO/AH) region relates to a resetting mechanism of body temperature (Hensel, 1973). Among these temperature-responsive neurons in the PO/AH region, the spontaneous activity of the warm-responsive neurons in which the firing rate increased with hypothalamic heating was markedly accelerated by iontophoretic application of 5-HT in rats (Hori and Nakayama, 1973) or rabbits (Murakami, 1973). Furthermore, 5-HT-containing fibers originating from cell bodies localized in the raphe nuclei of the midbrain, the pons and the medulla oblongata (Dahlström and Fuxe, 1964; 1965), project into the PO/AH area, the thalamus, the limibic system, the neocortex, and the neostriatum via the medial forebrain bundle in the lateral thalamus (Dahlström and Fuxe, 1964, 1965). These observations lead to a presumption that the serotonergic system in the brain stem may play an important role in the thermoregulatory mechanism in the CNS. Lysergic acid diethylamide (LSD) antagonized 5-HT excitation of single neurons in the brain stem of decerebrate cats (Boakes, Bradley, Briggs and Dray, 1970) and suppressed the activity of the raphe neurons in the brain stem (Weiss and Aghajanian, 1971). The purpose of the present experiments was to learn whether the depression of the serotonergic system by localized application of LSD affects thermoregulation in rabbits.

Key words: serotonergic system, thermoregulation, raphe nuclei, lysergic acid diethylamide, hyperthermia.

METHODS

Healthy male rabbits weighing between 2.6 and 3.5 kg were used in the experiments. Rectal, ear- and back-skin temperatures were continuously recorded with thermocouples (copper and constantan) connected to a precise potentiometer and pen recorder (25 R, Ookura Electric Co.). The rectal temperature was measured with a thermocouple probe covered with polyethylene tubing, inserted into the rectum 10–20 cm beyond the anus. For measuring the skin temperature, a thermocouple was placed with a collodion solution on the shaved skin surface. The oxygen consumption of the rabbits was monitored on an oxygen analyzer (Beckman F3), which continuously analyzed the oxygen concentration of the helmet effluent air, pulled by an electric pump with a flow of 8–10 l/min. Metabolic heat production was calculated

Table 1. Effects of LSD administration at various ambient temperatures

T_a (°C)	LSD (μg/kg)	Changes in T_{re} (°C)	Changes in T_{ear} (°C)
15	15.30 ± 1.17 (n = 4)	1.31 ± 0.35 (n = 4)	−1.12 ± 0.83 (n = 4)
25	12.77 ± 1.98 (n = 7)	1.93 ± 0.37 (n = 7)	−11.17 ± 0.35 (n = 4)
35	16.43 ± 1.72 (n = 3)	1.41 ± 0.60 (n = 3)	−6.67 ± 0.92 (n = 3)

in watts from the animal's oxygen consumption. A respiratory quotient (RQ) of 0.82 was assumed. One liter of oxygen consumed/hr was equivalent to a heat production of 5.597 W.

For the microinjection of drugs into brain tissue, a stainless-steel cannula (1.0 mm in diameter) with an obturator was implanted in rabbits anaesthetized with thiamyl sodium at an intramuscularly dose of 80–90 mg/kg. The tip of the cannula was placed on the nucleus raphe dorsalis (AP −11.5, L 0.0, H 16.0) or on the nucleus raphe magnus (AP −12.5, L 0.0, H 21.5), according to rabbit brain coordinates described by Sawyer, Everett and Green (1954). Subsequently, the cannula was fixed on the skull with dental cement. About 2 weeks were allowed for recovery. All microinjections of drugs were performed at the ambient temperatures of 15, 20, 25, 30 and 35°C after the rectal and skin temperatures of rabbits loosely restrained had reached a stationary value; this took usually 30–60 min.

After the end of the experiments, the animals were sacrificed with an over-dose of anaesthetic, perfused with a formaline–saline solution (10%) through the cardiac left-ventricle, and the brains were removed and placed in a formaline solution. The tip of the cannula was examined microscopically. All data from the animals in which the tip of the cannula was verified to be out of the desired nuclei was excluded in the present experiment. Lysergic acid diethylamide (LSD), 5-hydroxytryptamine (5-HT, Sigma), and an antipyretic (Sulpyrine, Daiichi Seiyaku Co.) were dissolved in sterilized saline.

RESULTS

Intravenous LSD

At ambient temperatures (T_a) of 15, 25 and 35°C, a rise in the rectal temperature (T_{re}) of rabbits was produced by the intravenous administration of LSD (12–17 μg/kg). A marked fall in the ear skin temperature (T_{ear}) also was obtained concomitantly. These data are summarized in Table 1. Changes in the rectal temperature and the ear temperature varied remarkably depending upon the ambient temperature. A reduction in the responses was obtained at the ambient temperature of 35°C. Attempts were made to lower the LSD-induced hyperthermia by the administration of antipyretic (Sulpyrine, 160 mg/kg) or 5-HT (3.0 mg/kg). These attempts were ineffective. It appears to be likely that the rise in the rectal temperature is not only due to an accumulation of heat but also due to the augmentation of heat production, because the rectal temperature increased remarkably even in cases with no changes in the ear temperature at the ambient temperature of 15°C. Changes in the

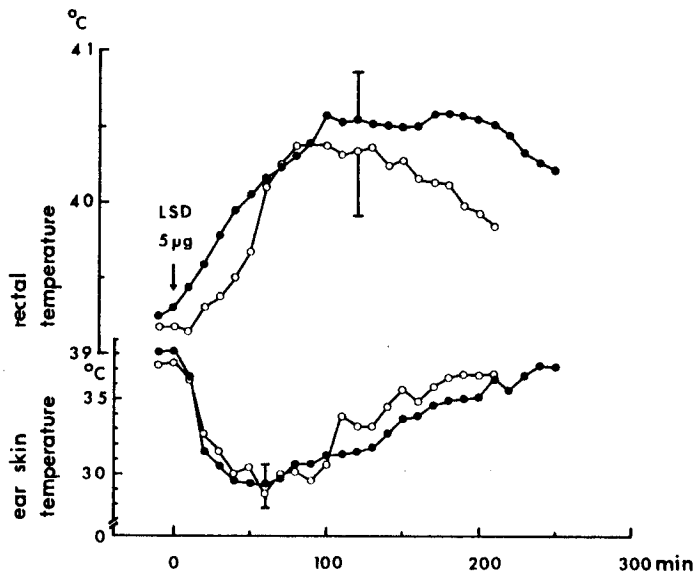

Fig. 1. Changes in rectal temperature and ear skin temperature with topical administration of LSD into the nucleus raphe dorsalis or the nucleus raphe magnus, at the ambient temperature of 29°C. Solid circles indicate mean values in 6 rabbits with the topical administration into the nucleus raphe dorsalis. Open circles indicate mean values in 5 rabbits with the topical administration into the nucleus raphe magnus. Bars represent the standard error of the mean at the time.

Table 2. Effects of LSD injected into the n. raphe dorsalis or the n. raphe magnus on rectal and skin temperature

Changes in T_{re} (°C)	Changes in T_{ear} (°C)	LSD (μg)	T_a (°C)	n
(A) *Microinjection of LSD into the N. raphe dorsalis*				
0.69 ± 0.13	−6.85 ± 0.79	1	29 ± 1	5
0.94 ± 0.16	−8.46 ± 1.10	5		6
1.60 ± 0.13	−9.50 ± 0.37	10		5
0.26 ± 0.11	−5.94 ± 1.21	1	20 ± 1	5
0.34 ± 0.04	−7.06 ± 1.53	5		5
0.58 ± 0.40	−7.78 ± 1.43	10		5
(B) *Microinjection of LSD into the N. raphe magnus*				
−0.12 ± 0.06	0.10 ± 0.26	1	29 ± 1	5
1.30 ± 0.36	−8.08 ± 0.84	5		5
1.26 ± 0.13	−8.04 ± 0.42	10		5

rectal and ear temperatures were smaller than those of rabbits in the ambient temperature of 25°C.

Local application of LSD into the raphe nuclei

Hyperthermia could also be evoked by means of direct injections of LSD into the raphe nuclei in the midbrain. There is a high concentration of 5-HT neurons in this area. Figure 1 shows a typical example of LSD-induced hyperthermia. Lysergic acid diethylamide (5×10^{-6} g/kg) was microinjected into the nucleus raphe dorsalis (NRD) of 6 rabbits or the nucleus raphe magnus (NRM) of 5 rabbits. This resulted in a rapid increase of rectal temperature of 1.95 and 1.65°C in the NRD and the NRM, respectively. This change was accompanied by marked vasoconstriction of the skin blood vessels even at the ambient temperature of 29°C. In the group of rabbits with LSD locally administered into the NRD, the average change of the rectal and ear temperatures was dependent on the LSD doses of 1, 5 and 10×10^{-6} g at both the ambient temperature of 29 and 20°C, (Table 2). Also, the mean change in the rectal and ear temperature at the ambient temperature of 20°C induced by each dose was less than that in the ambient temperature of 29°C. In the group of rabbits with LSD administered into the NRM, the results were almost similar to those of local LSD administration into the NRD except that a reduction of the responses was induced by the injection of 1 or 10 μg of LSD, even at the ambient temperature of 29°C.

Changes in heat production with local administration of LSD into the raphe nuclei

At a dose (5 μg) which induced similar changes in the rectal and ear temperatures both with application to the NRD or the NRM, LSD was injected into the raphe nuclei at 20°C ambient temperature. Heat production increased slightly in 3 rabbits with a cannula at the NRD as shown in Figure 2, while in four rabbits with a cannula at the NRM nothing occurred over the 180 min period following administration of the drug. Similar results were observed even at the 29°C ambient temperature. Thus, in a neutral or hot environment, LSD seemed to produce varying augmentations of heat production through the action of the NRD, in addition to a great inhibition of heat loss.

DISCUSSION

It was observed that local administration of LSD into the NRD or the NRM resulted in hyperthermic responses showing a fall in the ear temperature and an increase in the rectal temperature. This hyperthermic response seems to be the same as those observed with a systemic administration of LSD (Horita and Gogerty, 1958). A depression in heat loss and an augmentation in heat production, which are induced by the action of LSD on the raphe nuclei, must be the principal cause of the hyperthermic responses developed with a systemic administration of LSD.

In a previous report, it was revealed that in the midbrain of rabbits, warm-responsive neurons are concentrated in the raphe region as are the neurons in rats and cats, and that these warm-responsive neurons are influenced greatly by skin temperature or by the intravenous administration of LSD. Concerning raphe neuronal responses to changes in skin temperature of the cat, Dickenson (1977) showed that there are two populations of neurons in the raphe nuclei, cells responding to warming the skin and cold-responsive neurons. Since the activity of these neurons was unchanged by mid-collicular sections in which all descending influences from the forebrain were severed, their response can be attributed solely to thermal information from the skin. Hence, these neurons responsive to brain temperature and skin temperature have a high probability of being part of the serotonergic system because of their positive-response to LSD administration. This interpretation postulates that the LSD-induced hyperthermia may be due to a dysfunction of the 5-HT neuron system originating at the midbrain raphe nuclei. These results lead to the possibility that thermal information ascending in the somatosensory system is mediated partly by the 5-HT neuron before arriving at the hypothalamic thermoregulatory neurons. If the transient lack of warm signal propagation in the 5-HT neuron system is the principal cause of the hyperthermic response caused

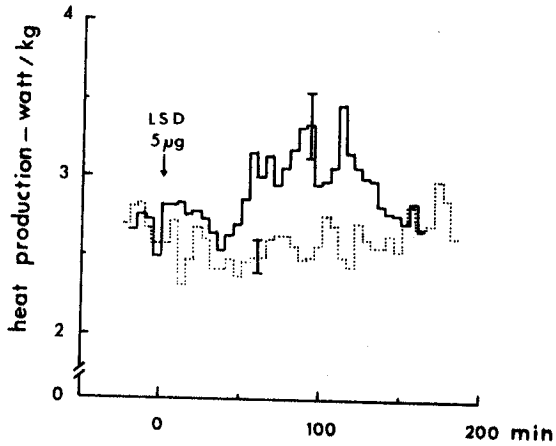

Fig. 2. Effects on heat production with LSD administered into the nucleus raphe dorsalis (solid line) or the nucleus raphe magnus (dotted line), at the ambient temperature of 29°C. The mean value is shown by the solid line (3 rabbits) and by the dotted line (4 rabbits) with the standard error of the mean (bars).

by LSD administration, it must be expected that the higher the ambient temperature, the greater the response. But the magnitude of the responses was lower than expected. At the ambient temperature of 15°C, the hyperthermic response was obtained without vasoconstriction, probably due to augmentation in heat production. As well, similar hyperthermic responses at both the ambient temperatures of 25°C and 35°C were obtained. This unexpectedly low hyperthermic response at the ambient temperature of 35°C may be due to the fact that heat production was already inhibited as much as possible by heat stimuli and only the reduction of heat dissipiation caused the LSD-induced hyperthermia. The physiological function of the thermal information propagated in the 5-HT neuron system still remains unknown.

Recently, it has been suggested that LSD may act as an agonist on the inhibitory 5-HT receptors in addition to being an antagonist at excitatory 5-HT receptors (Aghajanian, Haigler and Bloom, 1972). The present observations also suggest that LSD acts as an agonist at the inhibitory 5-HT receptor on the warm-responsive neuron receiving cold signals from the skin. Various doses of LSD were microinjected into the NRD or the NRM in the present experiments, which provided similar magnitudes of autonomic thermoregulatory responses. A slight increase of heat production was caused by a local administration of LSD into the NRD (Fig. 2). More temperature units responding to skin cooling have been observed in the NRM than in the NRD (Dickenson, 1977). This indicates that afferent cold inputs to the heat production system can be relayed at this nucleus. Hence, LSD administration into the NRM must affect this type of receptor antagonistically. The fact that at the ambient temperature of 29°C, a lower heat production was obtained with the administration of the drug into the NRM than into the NRD, implies that there is less of a hyperthermic response in the NRM than in the NRD.

From these results it could be concluded that the afferent input through cold fibers makes a synapse with 5-HT inhibitory receptors on the warm-responsive neurons in the midbrain. As well, there is a parallel connection with the excitatory synapse in the heat production system. Thus, LSD acts on both 5-HT inhibitory and excitatory receptors as an agonist and an antagonist, respectively, to induce the hyperthermic reaction.

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