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RAPID DEVELOPMENT OF TOLERANCE UPON CENTRAL INJECTION OF LSD

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Summary

LSD given into the lateral ventricle produces a dose-dependent rise in temperature accompanied by behavioral excitation. Tolerance to the pyretogenic effect rapidly develops upon intraventricular (ICV) injection of LSD following an intravenous injection of the same drug. Both Cinanserin and Cyproheptadine attenuate the pyretogenic response induced by LSD. Phenoxybenzamine attenuates the pyretogenic response induced by LSD to a greater degree than Dibozane.

Lysergic acid diethylamide (LSD) given either subcutaneously or intravenously produces hyperthermia and behavioral excitation in rabbits (1,2). A central mechanism of action is involved in the hyperthermia since cervical spinal section d-tuborcurarine and sodium pentobarbital will abolish this effect, while behavioral excitation can be abolished by lowering the level of brain norepinephrine by repeated administration of α-methyltyrosine in rabbits (3) and in rats (4). LSD influences the central nervous system in different aspects, such as the psychic, somatomotor, and autonomic nervous functions. (5).

The possibility that LSD may act by stimulating central tryptaminergic receptors by mimicking the action of endogenous serotonin (5HT) has been considered (6,7,8). Inhibition of the effects of 5HT by LSD has also been described (9,10). Jacob et al. (11) reported the possible existence of two phases in the hyperthermic action of 5HT, an early one relatively insensitive to LSD and a late one readily antagonized by this drug.

Gogerty and Dille (12) reported earlier that rabbits developed tolerance to the pyretogenic effect of LSD after daily parenteral administration of 50 µg for a period of four to five days. The duration of tolerance to a single dose lasted up to nine days.

The purpose of the present study was to differentiate the development of tolerance to the pyretogenic effect of LSD upon microinjection into the lateral and third ventricles following its intravenous injection. Also some pharmacological agents were used to attenuate the pyretogenic and behavioral responses.

Materials and Methods

Male New Zealand rabbits (Totem Farms, Kirkland, WA) weighing 2.3-2.8 kg

were used. A week prior to the experiment the rabbits were cannulated while under Equithesin TM (Jensen-Salsbery Lab) anesthesia with microinjection guide sleeve assembly units, consisting of a 21-gauge needle in a plastic mount. By use of the stereotaxic atlas by Monnier and Gangloff as reference (13), the guide sleeve was positioned at coordinates AP(-) 1.0 mm, L 1.5 mm, DV(-) 8.0 mm for the lateral ventricle and AP(-) 7.5 mm, L 0.0 mm, DV(-) 13.5 mm for the third ventricle. During the lowering of the assembly, dural bleeding was controlled with gel foam (Upjohn). The assembly was anchored by dental acrylic and stainless steel screws to the calvarium. Ventricular cerebrospinal fluid was mapped with a 26-gauge microinjection stylet attached to a 1-ml syringe via a length of teflon tubing.

On the day of the experiment, the animals were conditioned for 3 to 4 hr in open wooden stanchions (14). Colonic temperature was measured with a rectal thermistor probe (introduced 5-6 inches into the rectum) connected to a telethermometer (Yellow Springs Instrument) and an automatic recorder (Leeds and Northrup). At the end of the experiment, the exact depth of the microinjection was verified by use of a dye marker (15).

LSD, phenoxybenzamine hydrochloride (Smith Kline and French), dibozane (McNeil, d&-alpha-methyl-tyrosine methyl ester hydrochloride (Regis Chem. Co.), propranolol hydrochloride (Ayerst Lab.), cyproheptadine hydrochloride (Merck, Sharpe and Dohme), and cinanserin hydrochloride (E.R. Squibb and Sons) were prepared in bacteriostatic sodium chloride injection U.S.P. (Travenol Lab.). The intraventricular microinjection of LSD was set at 10 µl/30 sec by use of a 26-gauge microinjection stylet connected to a teflon tubing and a 1.0-ml syringe. A manually operated micrometer drive (Micrometric Instrument) was employed for the delivery of the injection. Dosage for the intraventricular injection (ICV) is expressed as per animal, and for the intravenous injection (IV) dosage is based on per kg of body weight.

Results

LSD at a dose of 2.5, 5, and 10 µg given into the lateral ventricle will elicit a dose-related rise in colonic temperature (Fig. IA), accompanied by behavioral excitation (Pupillary dilatation, increased locomotor activity, constriction of the ear vasculature, increased respiration and hyperalertness). The temperature peaks within 1 to 2 hr and gradually returns to basal temperature within 4 hr from the time of injection, except in the case of the 10 µg dose in which the effect lasted 6 hr. Behavioral excitation lasted for only 1-1/2 hr from the time of drug injection. Neither the picure from the injection stylet nor the injection of 10 µl of bacteriostatic saline effected any change in colonic temperature or behavior.

So that we could determine the development of pyretogenic tolerance upon microinjection of LSD into the lateral ventricle, we challenged animals that received 10 µl of saline, 2.5, 5.0 or 10.0 µg LSD ICV with an intravenous injection of 25.0 µg/kg of the same drug. Fig. IB shows that the normal pyretogenic response induced by 25 µg/kg IV of LSD was 1.35±0.15°C, whereas animals that received an ICV dose of LSD 4 hours prior to the IV injection exhibited responses that were markedly reduced. This was especially true in those animals that received the 5.0 µg of LSD ICV; the peak temperature response dropped to +0.4°C±0.1°C (Fig. 1B).

The administration of 5 µg LSD into the third ventircle produced a rise of +0.7°C±0.1°C in temperature; the peak was reached within 1-1 1/2 hours from the time of drug injection (Fig. 2A). The hyperthermia was also accompanied by behavioral excitation. When these animals were challenged with 25 µg/kg

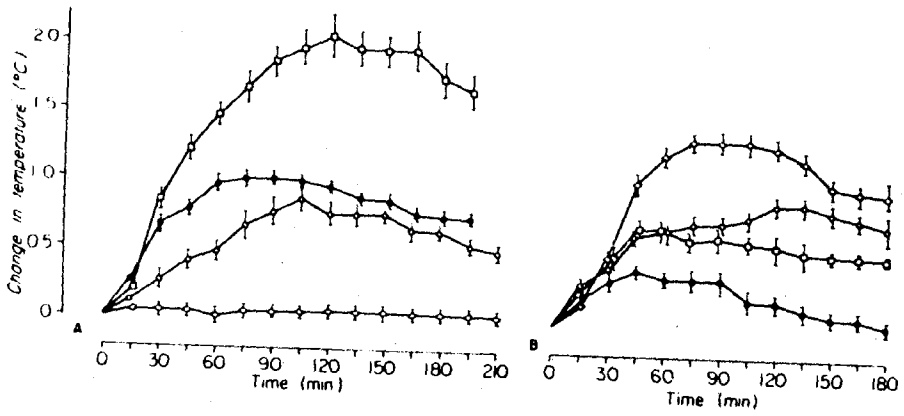

FIG. I

(A) Dose-response curve upon intraventricular injection of LSD into the lateral ventricle of rabbits:

◇, control saline 10 µl, (n=5), ○, 2.5 µg LSD, (n=6); ●, 5.0 µg LSD, (n=10); □, 10.0 µg LSD (n=6). Each curve represents the average colonic temperature responses of the individual animals. n represents the number of animals. Vertical lines represents SEM.

(B) Tolerance development upon intraventricular injection of LSD into the lateral ventricle 4 hr prior to the intravenous administration of 25.0 µg LSD: ◇, control saline, ICV, +25 µg LSD IV (n=5); ○, 2.5 µg LSD ICV, +25.0 µg LSD IV, (n=6); ●, 5.0 µg LSD ICV + 25.0 µg LSD IV, (n=10); □, 10.0 µg LSD ICV + 25.0 µg IV, (n=6). Each curve represents the average colonic temperature responses of the individual animals. Vertical line represents SEM.

LSD IV 4 hr after the ICV injection of 5 µg LSD, the temperature response was reduced from +1.35°C to +0.7°C (Fig. 2B).

Pretreatment of the animals with two antiserotonergic drugs, cyroheptadine (1.0 mg/kg IV) and cinanserin (10 mg/kg IV), prior to the ICV injection of 5 µg LSD into the lateral ventricle produced a significant reduction in the pyretogenic response, as compared with untreated animals (Fig. 3). However, the behavioral excitation induced by ICV injection of LSD was not altered by these drugs.

Phenoxybenzamine (5.0 mg/kg IV) given 2 hr prior to the ICV injection of 5 µg LSD partially attenuated the pyretogenic but not the behavioral response. Dibozane, another α-blocker, at a dose of 5.0 mg/kg IV attenuated the pyretogenic response to a lesser degree than phenoxybenzamine (Fig. 4). Both the phenoxybenzamine and dibozane in control animals produced mild sedation and slight hypothermia.

Discussion

A rapid development of tolerance to the pyretogenic effect of LSD is produced when the drug is administered by the intraventricular route. In contrast, with parenteral administration, it requires at least four to five days

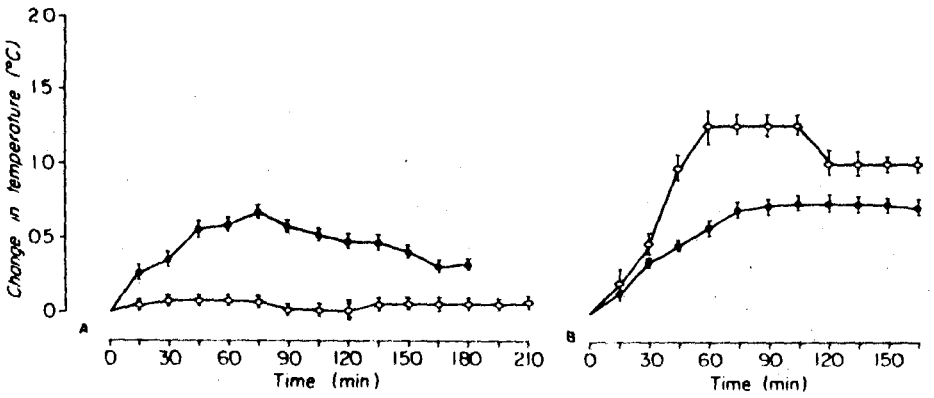

FIG. 2

(A) Change in colonic temperature upon intraventricular injection of 5.0 µg LSD into the third ventricle of the rabbits. ◇, control saline 10 µl, (n=5); ● , 5.0 µg LSD, (n=7). Each curve represents the average colonic temperature responses of the individual animals. The n represents the number of animals. Vertical lines represents SEM.

(B) Tolerance development upon intraventricular injection of 5.0 µg LSD into the third ventricle 4 hr prior to an intravenous dose of 25.0 µg LSD. ◇, control saline 10 µl ICV + 25.0 µg LSD IV, (n=5); ● , 5.0 µg LSD ICV + 25.0 µg LSD IV, (n=7). Each curve represents the average colonic temperature responses of the individual animals. N represents the number of animals. Vertical lines represent SEM.

before partial tolerance can be achieved. Tolerance to the LSD-induced behavioral excitation was not observed. The rise in temperature upon intraventricular injection of LSD is dose-dependent and is accompanied by behavioral excitation. Microinjection of 5.0 µg LSD into the lateral ventricle produces a slightly higher degree of hyperthermia than is obtained with the third ventricle; this may be due to the lateral ventricle being located nearer to the preoptic region of the anterior hypothalamus, which is believed to be the thermoregulatory center in the central nervous system (16).

The attenuation of the pyretogenic response produced by pretreatment of serotonergic blockers prior to the ICV injection of LSD supports the hypothesis that LSD mimics the action of endogenous serotonin, since experimental observations in our laboratory show that ICV injection of 150 µg 5HT to rabbits pretreated with the same dose of cyproheptadine and cinanserin will attenuate the pyretogenic response by 60-70%.

The α-blockers phenoxybenzamine and dibozane also attenuate the pyretogenic response of ICV LSD; this effect is a strong indication of the interaction of LSD with catecholamine receptors in the central nervous system. The pyretogenic response to norepinephrine at a dose of 100 µg given into the lateral ventricle of the rabbits was also attenuated by these α-blockers. The β-adrenergic blocking agent propranolol did not alter either the pyretogenic nor the behavioral response induced by intraventricular injections of LSD.

that the pyretogenic response induced by intraventricular injection of LSD is mediated by its action on both 5-HT receptors and adrenergic α-receptors. Studies are now underway to determine the exact mechanism of its action by intracerebral injection of the drug into the different areas in the central nervous system where these amines are present.

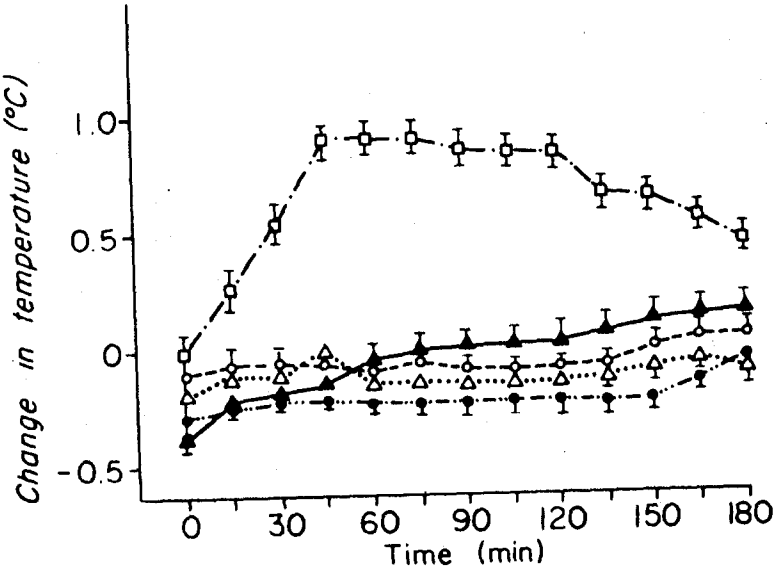

FIG. 3

The effect of serotonergic blockers cyproheptadine and cinanserin given intravenously prior to the intraventricular injection of 5.0 µg LSD into the lateral ventricle. □, 5.0 µg LSD ICV, (n=10); ○, cinanserin control 10 mg/kg IV 20 min prior to 10 µl saline; (n=5); △, cinanserin 10 mg/kg IV 20 min prior to 5.0 µg LSD ICV, (n=5); ●, cyproheptadine control mg/kg IV 2 hr prior to 10 µl saline, (n=3); ▲, cyproheptadine mg/kg IV, 2 hr prior to 5.0 µg LSD ICV, (n=5).

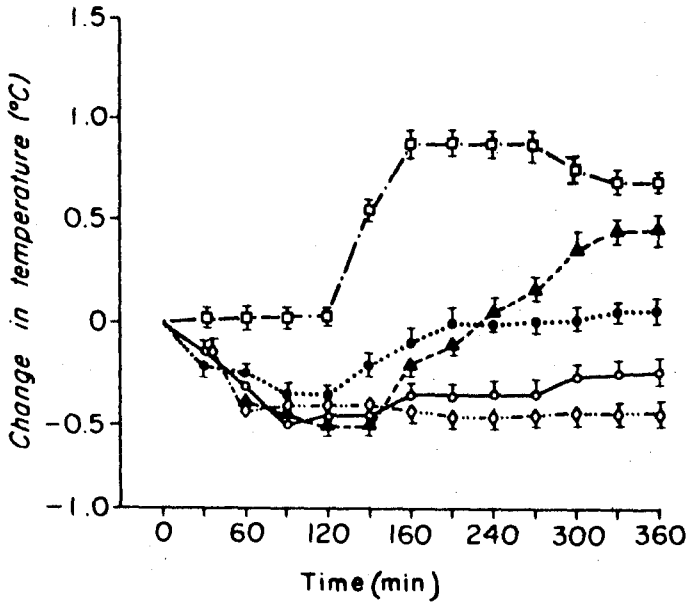

FIG. 4

The effect of alpha blockers phenoxybenzamine and dibozane given intravenously 2 hr prior to the intraventricular injection of 5.0 µg LSD into the lateral ventricle. □, intravenous saline + 5.0 µg LSD ICV, (n=5): ◇, Dibozane 5.0 mg/kg IV + 10 µl saline ICV (n=3); ◆, Dibozane 5.0 mg/kg IV + 5.0 µg LSD ICV, (n=5); ○, phenoxybenzamine 5.0 mg/kg IV + 10 µl saline; ●, phenoxybenzamine 5.0 mg/kg IV + 5.0 µg LSD ICV, (n=5). Each curve represents the average colonic temperature responses of the individual animals. N represents the number of animals. Vertical line represents SEM.

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