ELICITING CONDITIONED TASTE AVERSION BY COBRA VENOM NEUROTOXIN IN RATS

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Summary: An attempt is made to study conditioned taste aversion(CTA) using cobra venom antivenom or lithium chloride as the Unconditioned Stimulus(US). Twenty-four hour water deprived rats were habituated for two consecutive days to drinking tap water in the drinking box for 15 minutes daily. On 3rd day they were allowed to drink 0.1% sodium saccharin. Thirty minutes later, they were injected with cobra venom (45 μg), antivenom (0.022 *ml*), antivenom followed by venom, lithium chloride (0.15 M, 4% body weight) or physiological saline. After two days of recovery the animals were water deprived for twenty four hours and water intake was measured on the 7th and 8th day. Retention test on the 9th day shows reduced saccharin consumption in the lithium chloride and venom groups. CTA was significantly reduced in the antivenom-venom group and absent in the antivenom and control group. It is concluded cobra venom can induce clear-cut CTA in rats.

Key words: rat sodium saccharin cobra venom antivenom lithium chloride conditioned taste aversion

INTRODUCTION

With the increased experimental interest in conditioned taste aversion the list of the agents producing CTA has been greatly expanded. During two decades of reasearch the agents used as the US included X-irradiation (12), lithium chloride (15), apomorphine (10), d-amphetamine and mescaline (5), ethanol (14), Cyclophosphamide (11), hypertonic saline (13), p-tetrahydrocannabinol (8), morphine and chlordiazepoxide (6), cyclohexamide (2), formalin (18), methyl mercury (3), anesthetics (4), intravenously injected isotonic saline (17). The present paper describes as attempt to elicit CTA by the cobra neurotoxin and to block its effects by the antivenom.

MATERIALS AND METHODS

Eighty Swiss strain albino rats, aged 3 months and weighing 150 to 200 *gms* of both sexes were used. The animals were bred in the laboratory of this college. They were housed 8 per cage with water and food freely available. Water was removed from the home cages 24 hr before the start of experiment. The rats were maintained on 24 hr water deprivation schedule throughout the experiment.

The drinking box was a plastic enclosure (30x22x17 cm) with a hole at the top permitting insertion of 50 *ml* glass drinking burette. The drinking spout was 7 cm above the floor level. The burette was filled either with tap water or 0.1% sodium saccharin. The volume of the fluid consumed was measured with 0.1 *ml* accuracy. The water deprived rats were allowed to drink tap

water for 15 min on two consecutive days. On the 3rd day all animal drank 0.1% sodium saccharin and were then divided into five groups: Group V received a subcutaneous injection of 45 μg of cobra venom neurotoxin 30 min after saccharin. Group A was subcutaneously injected with 0.022 *ml* of the antivenom (polyantivenin) 30 min after saccharin intake. Group AV injected fifteen min after saccharin intake 0.022 *ml* of polyantivenin and 15 min later 45 μg of venom. Group LiCl received 30 min after saccharin intake an intraperitoneal injection of 0.15 M LiCl 4% body weight. Group C the control group received 30 min after saccharin intake a intraperitoneal injection of physiological saline (4% body weight).

After injection the rats were returned to home cages for 48 hours with water and food freely available. On the 6th day they were put on the 24-hr water deprivation schedule again and water intake was measured on the 7th and 8th day. Retention test was conducted on the 9th day.

Appropriate dose of neurotoxin was determined in preliminary experiments on additional 18 rats. The venom was of *Naja naja* species and was supplied in the concentration of 50 $\mu g/ml$ Injection of 80 μg caused 100% death, 65 μg resulted into 50% survival, and with 45 μg there was 100% survival. All rats not surviving died within 24 *hr*. The polyantivenom used in this experiment was of such an intensity that one *ml* of antivenom can neutralize 2.5 to 3 *mg* of venom. Rats were injected with 0.022 *ml* of antivenom providing full protection against the 45 μg of venom.

RESULTS

The results are summarized in Table I. In all groups fluid consumption increased on the two first days and became assymptotic on day 3. Water consumption on days 7 and 8 was not statistically different from day 2. Saccharin intake during the retention test on day 9 dropped to

TABLE I: Fluid consumption (*ml*) on subsequent days of the experiment in various experimental and control groups.

	N	*W(1)*	*W(2)*	*S(3)*	*W(7)*	*W(8)*	*S(9)*	*D9-D-3*
Venom	16	4.4±.36	6.38±.60	6.67±.52	6.0±.82	6.1±.67	3.7±.25	—3.0± 0 40
Antivenom	16	6 0±.92	8.36±.92	8.68±.90	9.38±.1.0	8.68±1.0	10.5±1.4	+1.8±.0.80
Antivenom Venom	16	5.84±.55	7.83±.50	8.18±.60	10.36±.85	9.63±.77	7.4±.50	—0.8±.0.32
Saline	16	6.63±.97	8.43±.62	8.53±.62	8.43±.70	8.43±.87	8.79±.85	+26±0.60
Lithium Chloride	16	5.36±.35	5.77±.32	6.68±.25	6.53±.42	6.69±.37	3.9±.27	—2.8±.0.27

about 50% of the saccharin volume consumed on day 3 in the venom group ($df = 15$, $t = 7.15$, $P < 0.001$) and in the poisoned control group LiCl ($df = 15$, $t = 10,2$, $P < 0.001$). Increase of saccharin intake on day 9 was not significantly dtfferent in the antivenom group A and unpoison-

ed group C ($df = 30$, $t = 1.5$, $P > 0.05$). Reduction of saccharin intake in the group AV, protected against poisoning by the antivenom injection was statistically significant ($df = 15$, $t = 2.46$, $P < 0.05$) but was significantly smaller than in the groups V ($df = 30$, $t = 4.3$, $P < 0.001$) and LiCl ($df = 30$, $t = 4.8$, $P < 0.01$).

DISCUSSION

There are great differences among drugs used in producing learned taste aversion, and there is no simple relationship between CTA strength and the intensity of the sickness as indicated by behavioural symptoms (16,9). NaCN (4 *mg/kg*) does not elicit aversion to 15% sucrose injected immediately prior to poisoning. Gellaminetriethiodide has failed to produce CTA in dosages causing muscle paralysis. Strychnine and warfarin are toxic to the rats but are not effetive in producing conditioned taste aversion. Sodium malonate failed to induce CTA when applied in a dose which causes 90% reduction of cerebral respiration. The highly toxic thallium produces only slight aversion. Copper sulphate, red squill and sodium fluoroacetate produce severe toxicosis and taste aversion. Nachman and Hartley (16) found that one of the most effective drugs in producing CTA is sodium fluoroacetate which affects cardiac, respiratory and nervous function rather than the gastrointestinal system. The venom of cobra *Naja naja* is primarily neurotoxic and the death is due to respiratory failure caused by paralysis of the respiratory centre. Dubois and Geiling (7) reported that cobra neurotoxin produces both convulsion and paralysis. Cardiovascular depression manifested by sweating, cold extremities and hypotension can be due to carbon dioxide retention and acidosis caused by respiratory failure.

On the other hand the first symptoms of the cobra bite are nausea and vomiting, sometimes also abdominal pain (1). It is probable that the CTA observedin the present experiments is due to this initial malaise rather than to the later interference with respiratory and cardiovascular function.

The possibility to prevent the venom elicited CTA by preceding antivenom injection indicates that venom-antivenom interaction extends to the changes responsible for CTA learning. Further experiments should examine the dynamics of this interaction in more detail, for inst. by injecting the antivenom at different intervals after administration of the venom.

The capability of sublethal dosages of cobra neurotoxin to elicit CTA is an important toxicological characteristic of this venom which may help to differentiate it from various toxins isolated from plants and animals.

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