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Failure to Elicit Conditioned Taste Aversion by Severe Poisoning

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IONESCU, E. AND O. BUREŠOVÁ. *Failure to elicit conditioned taste aversion by severe poisoning.* PHARMAC. BIOCHEM. BEHAV. 6(3) 251–254, 1977. – In an attempt to assess the universal validity of the conditioned taste aversion (CTA) paradigm, various types of poisoning (UC) were associated with the gustatory CS. Water deprived rats were habituated for two days to the drinking box, where water was available for 15 min. On Day 3, access to the CS (0.1% saccharin 15 min) was followed after 30 min by a sublethal dose of the poison (0.15 M LiCl, 4% body weight; 0.1 M sodium malonate, 1% body weight; pyrrolopyrimidine drug BW 58-271, 15 mg/kg; sodium cyanide 4 mg/kg; sodium iodoacetate 40 mg/kg; sodium fluoride 30 mg/kg; gallamine triethiodide 40 mg/kg). Rats injected with the last drug were maintained under artificial respiration until muscular paralysis disappeared. After 4 days of recovery, water deprivation schedule was resumed on Days 8 and 9. During the retention test on Day 10 saccharin consumption dropped by 60% in the LiCl poisoned rats, but no CTA developed in animals poisoned by pyrrolopyrimidine, gallamine, malonate and cyanide. CTA of intermediate intensity was evoked by iodoacetate and fluoride. The absence of CTA was not due to the amnesic effect of poisoning, since LiCl administration to NaCN poisoned rats produced CTA of usual intensity. It is concluded that CTA is not related to the overall severity of poisoning but rather to the effect of the poison on specific interoceptors.

Learning Memory Consolidation Oxidation metabolism Glycolysis Anoxia Spreading depression

RESEARCH into the conditioned taste aversion (CTA) concentrated so far on treatments which can serve as a reliable US. The bulk of CTA studies (for bibliography see Riley and Baril [22]) employed x-irradiation, LiCl or apomorphine injections to induce aversion to flavors ingested within several hours before the onset of sickness. Other, less frequently used poisons and treatments eliciting the presumable sickness are cyclophosphamide [11], cyclohexamide [3], formaline [26] or vestibular stimulation [13]. CTA can also be induced by a number of psychoactive drugs, including morphine, chlordiazepoxide, scopolamine, amphetamine and various anesthetics (for review see Vogel [24]) at concentrations which cause no obvious adverse effects or are even reinforcing. Whereas these results indicate that CTA can be produced without obvious poisoning, it is less clear whether all poisons can produce CTA. Berger [1] failed to elicit CTA using strychnine (1 mg/kg) as the US and similar failure was reported by Millner and Palfai [16] for metrazol. Nachman and Hartley [19] compared various rodenticides for their effectiveness in eliciting CTA to sucrose offered 2–4 min before administration of the poison. Only sodium fluoroacetate and copper sulfate elicited CTA comparable with that caused by LiCl. Similar dosages (50% of the lethal dose) of warfarin, strychnine, and sodium cyanide were ineffective whereas thallium sulfate and red squill had intermediate effects. The absence of CTA is not surprising when the symptoms of poisoning appear after a delay exceeding 24 hr (warfarin, thallium sulfate) but is difficult to explain in case of rapidly acting poisons like NaCN, strychnine sulfate and metrazol. It can be surmised that these drugs produce visceral symptoms of poisoning, but that formation and consolidation of CTA engram is prevented by seizure activity and brain anoxia. An alternative possibility is that CTA does not encompass all types of poisoning and that the above drugs belong to a class of stimuli which cannot serve as US in the CTA paradigm.

It is obvious that comparison of poisons which do or do not elicit CTA can provide important information about the nature of the US and contribute to elucidation of CTA mechanisms. The present paper describes an attempt to elicit CTA by poisons causing muscular paralysis (gallamine-triethiodide), and spreading depression (BW 58-271) or interfering with tissue respiration (malonate, NaCN) and glycolytic processes (NaF, iodoacetate)

METHOD

Animals

The experiments were performed in 98 male hooded rats (Druckrey strain) aged 3 months. The animals were housed in group cages (6 animals per cage) with food and water freely available. Water was removed from the home cage 48 hr before the experiment and the animals were maintained on a 24-hr water deprivation schedule during the actual testing.

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Apparatus

The drinking box was a plastic enclosure (17 × 49 × 30) to the front wall of which was attached a 25 ml pipette with the spout 6 cm above the grid floor. The pipette was filled either with tap water or 0.1% sodium saccharin. The volume of the fluid consumed was measured with 0.1 ml accuracy.

Procedure

In a series of preliminary experiments the lethal doses of the drugs used were checked and the toxicity levels used in the actual experiments were set so that mortality did not exceed 30 to 40%. The rats were allowed 15-min access to water in the drinking spout on Days 1 and 2. On Day 3 saccharin solution (0.1%) was used as the test fluid.

In Experiment 1 animals were randomly assigned to treatment groups which were injected 30 min later with NaCl (0.15 M, 4% body weight), LiCl (0.15 M, 4% body weight), NaCN (4 mg/kg), pyrrolopyrimidine drug BW 58-271 (15 mg/kg), gallaminetriethiodide (40 mg/kg), sodium iodoacetate (40 mg/kg), sodium fluoride (30 mg/kg), and sodium malonate (0.1 M, 1% body weight). The rats were then returned to their home cages. Rats injected with gallaminetriethiodide were placed on arteficial respiration (60/min, open system) which was administered through a face mask until the muscle paralysis was over (usually one or two hr).

In Experiment 2 saccharin drinking was followed after 15 min by injection of NaCN (4 mg/kg). When the symptoms of poisoning were fully developed 15 min later the animals received an additional injection of LiCl (0.15 M, 4% body weight). Another group was injected 30 min after saccharin intake with LiCl (0.15 M, 4% body weight) followed 15 min later by NaCN (4 mg/kg).

On Days 4, 5, 6 all animals had free access to water in their home cages. Water was removed on Day 7 and the 24-hr water deprivation schedule was resumed on Days 8, 9, and 10. Consumption of water was tested in the drinking box on Days 8 and 9 and saccharin intake was measured on Day 10.

Statistical analysis of data was performed using Student's t-test for paired values and ANOVA. Only rats which passed through all stages of the experiment were used in the final evaluation.

RESULTS

Experiment 1

Average fluid consumption in various phases of the experiment is shown in Table 1. Analysis of variance showed no statistically significant differences between groups on Days 1 and 2, $F(7, 70) = 1.1$, $p>0.05$, and 3, $F(7, 70) = 1.1$, $p>0.05$. Water consumption on Day 8 was slightly reduced in the gallaminetriethiodide and malonate groups probably because of residual sickness or generalized avoidance of fluids in the drinking box. On Day 9 all groups consumed volumes of water which were not significantly different from the water consumption on Day 2. The difference observed on Day 10 could be considered, therefore, as a specific manifestation of the conditioned saccharin aversion.

Saccharin consumption was slightly higher on the test day than on the treatment day in the NaCl control group, but the difference was not statistically significant. Similar results were obtained with cyanide, pyrrolopyrimidine, gallaminetriethiodide, and malonate. Neither of these drugs elicited any appreciable reduction of saccharin intake on Day 10 in comparison with Day 3 consumption in the same group. Strong CTA was seen in the LiCl group in which saccharin intake on the testing day dropped below 40% of saccharin intake on Day 3 ($t = 6.3$, $df = 9$, $p<0.001$). Intermediate saccharin aversion developed in the iodoacetate and fluoride groups where CTA was statistically significant ($t = 2.3$, $df = 8$, $p<0.05$ for iodoacetate and $t = 2.7$, $df = 9$, $p<0.05$ for fluoride). Analysis of variance was applied to the differences in saccharin consumption on Days 3 and 10. The LiCl group was significantly different from all other groups, $F(1, 73) = 34.7$, $p<0.001$, and the sodium chloride and iodoacetate groups were different from the remaining groups other than LiCl, $F(1, 73) = 16.2$, $p<0.001$. On the other hand, no difference was found between the iodoacetate and fluoride groups, $F(1, 73) = 0.1$, n.s., and between the NaCl and the NaCN, pyrrolopyrimidine, gallaminetriethiodide, and malonate groups, $F(1, 73) = 0.2$, n.s.

Experiment 2

Figure 1 compares the NaCN and LiCl groups of Experiment 1 with the two groups of Experiment 2. Analysis of variance showed no significant difference

TABLE 1

THE EFFICIENCY OF VARIOUS POISONS IN ESTABLISHING THE CONDITIONED TASTE AVERSION TO SACCHARIN

Substance	n	Fluid consumption on Days 1(W)	2(W)	3(S)	8W)	9(W)	10(S)	D 10-D 3 difference
Lithium chloride	10	8.7 ± 0.6	9.8 ± 0.4	10.1 ± 0.5	8.2 ± 0.7	9.6 ± 0.7	3.8 ± 0.7	−6.3 ± 1.0
Pyrrolopyrimidine	10	6.3 ± 0.8	7.6 ± 0.9	8.5 ± 0.6	8.6 ± 0.3	9.0 ± 0.4	8.2 ± 1.1	−0.3 ± 0.8
Gallaminetriethiodide	9	7.8 ± 0.9	9.6 ± 0.9	8.8 ± 1.4	7.8 ± 0.9	9.4 ± 1.2	8.5 ± 1.4	−0.3 ± 0.9
Sodium cyanide	10	7.8 ± 0.9	8.7 ± 1.2	9.0 ± 0.5	9.5 ± 0.7	10.5 ± 0.4	9.3 ± 1.2	+0.3 ± 0.6
Sodium malonate	10	8.3 ± 0.5	9.2 ± 0.3	8.8 ± 0.5	4.8 ± 0.8	7.7 ± 0.8	9.4 ± 0.6	+0.6 ± 0.7
Sodium iodoacetate	9	8.8 ± 0.5	10.1 ± 0.5	10.0 ± 0.7	10.5 ± 0.6	10.3 ± 0.8	7.2 ± 1.4	−2.8 ± 1.2
Sodium fluoride	10	9.0 ± 0.6	9.6 ± 0.7	10.2 ± 0.9	10.9 ± 1.1	11.6 ± 1.0	8.5 ± 1.5	−2.7 ± 1.0
Sodium chloride	10	9.1 ± 0.7	9.8 ± 0.8	10.3 ± 0.6	9.3 ± 0.7	10.2 ± 0.8	10.7 ± 1.0	+0.5 ± 0.7

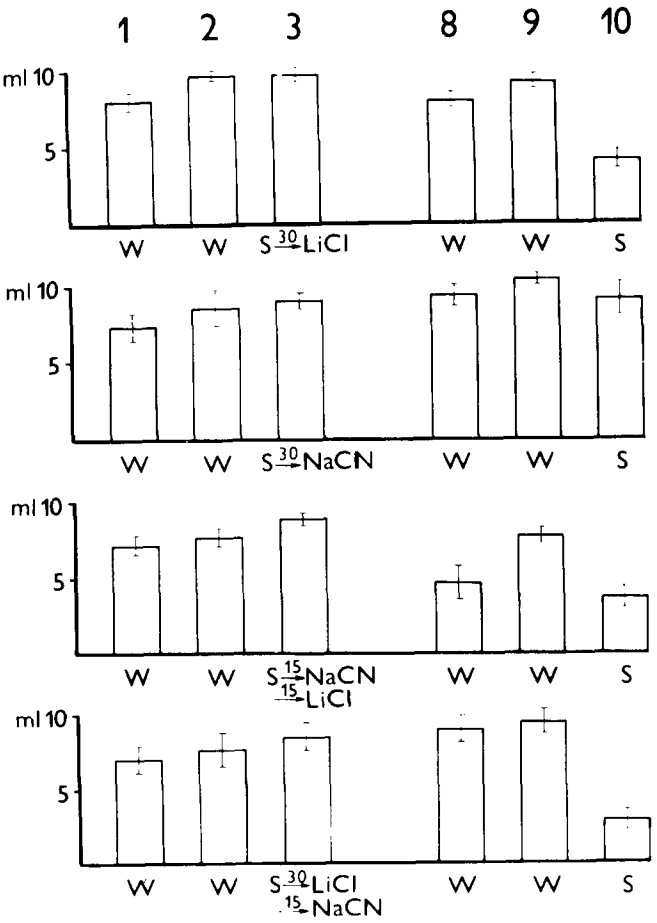

FIG. 1. High dosage of sodium cyanide (4 mg/kg) which does not elicit CTA, does not prevent CTA formation when injected 15 min before or 15 min after LiCl administration. Ordinate: Fluid consumption. Abscissa: Days of the experiment. W – water, S – saccharin, LiCl or NaCN – injection of lithium chloride or sodium cyanide. Horizontal arrows denote the time between the end of saccharin drinking and drug injection or between injections.

between these groups on Days 1–3. $F(3, 36) = 1.53$, $p>0.05$, for saccharin intake on Day 3. Water intake was significantly lower on Day 8 than on Day 2 in the NaCN – LiCl group ($t = 2.3$, $df = 9$, $p<0.05$), but this difference disappeared on Day 9 when water consumption was not significantly different from the water consumption on Day 2 in any group. Saccharin intake on Day 10 showed marked CTA not only in the LiCl group but also in the NaCN – LiCl and LiCl – NaCN groups, in which the LiCl effect was either superimposed on the NaCN poisoning or was followed after a brief interval by histotoxic anoxia. Saccharin consumption on Day 10 was significantly lower in the LiCl treated groups than in the group poisoned with NaCN alone, $F(1, 36) = 37.1$, $p<0.01$. On the other hand, the LiCl group was not significantly different from the LiCl – NaCN and NaCN – LiCl groups, $F(1, 36) = 0.6$, n.s. and there was no significant difference between the latter two groups, $F(1, 36) = 0.7$, n.s. Aversion was also demonstrated by the statistically significant differences between saccharin consumption on Days 3 and 10 in the LiCl, LiCl – NaCN and NaCN – LiCl groups ($t = 6.0$, $df = 9$, $p<0.01$; $t = 5$, $df = 9$, $p<0.01$; $t = 5.2$, $df = 9$, $p<0.01$).

DISCUSSION

The above results confirm the report by Nachman and Hartley [19] that severe intoxication with NaCN (2 mg/kg) does not elicit aversion to the sweet fluid (15% sucrose) ingested immediately prior to poisoning. In spite of the high dosage of NaCN (4 mg/kg) which caused death of 27% rats in the present study, no CTA was observed in the surviving animals. As NaCN causes brain anoxia resulting into depression of EEG activity, and depolarisation of cerebral cortex [4], the possibility must be taken into account that the peripheral symptoms of poisoning are not registered in the higher levels of the CNS. This explanation was ruled out by the outcome of Experiment 2, demonstrating that NaCN poisoning does not diminish the effectiveness of LiCl solutions administered 15 min before or 15 min after NaCN injection. The fact that short-term storage of the gustatory trace and its association with the visceral signals of poisoning can proceed during deep depression of neural functions has been demonstrated for spreading depression [5,7], anesthesia [5, 16, 23], hypothermia [14], and strychnine seizures [19]. The obvious implication is that formation of the permanent CTA engram proceeds at brain levels the activity of which is not critically impaired by the above treatments. It seems that the underlying mechanisms are related to homeostatic regulations maintained by lower brain centres even in deep coma.

The applied dose of the pyrrolopyrimidine derivative BW 58-271 elicits general anesthesia accompanied by waves of cortical spreading depression [9, 20, 21]. Contrary to the report by Winn *et al.* [25] who induced CTA by application of 12% KCl to cerebral cortex of rats, the pyrrolopyrimidine experiments confirm the finding that CSD neither elicits CTA [2, 5, 6, 18] nor prevents the association of the short-term gustatory trace with poisoning [7]. Unlike anesthesia CSD decreases persistance of the short-term gustatory trace and may impair CTA acquisition when maintained during prolonged CS-US intervals [6].

Muscular paralysis evoked by gallaminetriethiodide would be fatal within several minutes but adequate artificial respiration prevents the development of asphyxia. Under these conditions brain functions are normal and symptoms of poisoning are restricted to skeletal muscles. As classical and operant conditioning has been demonstrated in curarized animals [10], the failure of gallaminetriethiodide to induce CTA is not due to impaired learning but rather to the lack of physiological effects necessary for CTA formation. In this respect muscle paralysis resembles footshock [12] and other nonvisceral stressful stimuli which also fail to evoke CTA. On the other hand, lack of obvious visceral symptoms does not preclude the effectiveness of poisoning. Nachman and Hartley [19] found that one of the most effective drugs in producing CTA is sodium fluoroacetate which affect cardiac, respiratory and nervous functions rather than the gastrointestinal system. The metabolic effects of fluoroacetate resemble those of cyanide: both poisons interfere with energy metabolism but the former inhibits earlier stages of the Krebs' cycle (inhibition of aconitase by fluoroacetate), whereas the latter blocks the oxidative enzymes. In accordance with this finding another inhibitor of oxidative metabolism, sodium malonate, failed to induce CTA when applied in a dose which caused 90% reduction of cerebral respiration [17]. On the other hand sodium fluoride and sodium iodoacetate, that is, poisons

primarily interfering with glycolytic processes [15], elicited significant CTA. It is conceivable that the visceral signals of poisoning are generated by interoceptors which are considerably resistant to anoxia but are affected by interference with glycolysis.

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