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The Taste Aversion Induction Properties of Two Long Duration Barbiturates[1,2]

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WAYNER, E. A., G. SINGER, M. J. WAYNER AND F. C. BARONE. *The taste aversion induction properties of two long duration barbiturates*. PHARMAC. BIOCHEM. BEHAV. 12(5) 807–810, 1980.—The ability of sodium phenobarbital (60 mg/kg) and sodium barbital (80 mg/kg) to produce a taste aversion in 23 hr fluid deprived rats was examined using a discrimination or two bottle taste aversion task (0.125% sodium saccharin solution or water). The interaction of both barbiturates with the effects of 3.0 mEq/kg lithium chloride (LiCl) was also examined. Results indicate that phenobarbital treatment alone produces a stronger saccharin aversion than does barbital. Also, barbiturate treatment 24 hr after LiCl administration does not attenuate saccharin avoidance, although phenobarbital treatment following LiCl administration was sufficient to induce a maximum aversion that did not extinguish after twenty days of continuous discrimination testing. These data suggest that the aversion inducing properties of the two barbiturates are dissimilar and that phenobarbital is the more effective agent in the production of saccharin aversion. In addition, barbiturate induced attenuation of conditioned taste aversion is apparently related to the periodic forced intake test model since it does not occur when a water and saccharin choice is available.

Barbiturates LiCl Saccharin aversion Sodium barbital Sodium phenobarbital Taste aversion

RECENTLY, it has been reported that either 60 mg/kg sodium phenobarbital [3,4] or 80 mg/kg sodium barbital [10] can significantly attenuate LiCl induced saccharin aversion when administered to fluid deprived rats prior to drinking on the first test day 72 hr after conditioning. This effect has also been reported for sodium pentobarbital, 15 mg/kg [4] or 9 mg/kg [10]. In addition, phenobarbital also attenuates X-irradiation induced taste aversion [5]. The mechanisms involved in this attenuation effect on taste aversion are unknown and do not appear to be related to the duration of barbiturate action or their dipsogenic properties [4,10].

In addition, the studies reporting an attenuation effect of the barbiturates also report a decrease in saccharin consumption by fluid deprived rats on test days subsequent to barbiturate treatment which is greater than the decrease produced by LiCl alone [4,10]. This effect has been suggested to be the result of barbiturate induced taste aversion, as hypnotic drugs are effective in reducing periodic forced consumption of a variety of fluids [8] including saccharin and ethanol solutions [11]. However, administration of sodium phenobarbital in conjunction with LiCl apparently results in a synergistic interaction of the two aversive compounds in the production of long term sapid fluid avoidance. Thus, the decrease in saccharin intake observed on post-barbiturate test days after LiCl in the previous studies [3, 4, 10] might either be the result of a barbiturate induced taste aversion or a synergistic interaction with the effects of LiCl.

The present study was designed to determine the effects of phenobarbital or barbital (60 mg/kg or 80 mg/kg, respectively) on LiCl induced saccharin aversion when administered only 24 hr after LiCl on the first test day after conditioning. The relative efficacy of both barbiturates in the production of taste aversion when administered alone was also examined. Thus, the dipsogenic [7, 9, 12] and the aversion inducing properties of the drugs alone or when administered to induce a synergistic interaction with the effects of LiCl were determined. A continuous discrimination or two bottle choice, saccharin solution or water, was utilized to obtain a more sensitive measure of the strength and duration of the saccharin aversion [2]. The two bottle task also allowed an examination of the specificity of the taste aversion attenuating effects of phenobarbital and barbital to the forced extinction model.

METHOD

Animals

Thirty-six Wistar derived male albino rats, 90–120 days old and 300–350 g, were individually housed in wire mesh cages (33×20×23 cm), and maintained on a 12 hr light/dark cycle at constant temperature (21°C) and humidity. They

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were randomly assigned to six groups with six rats in each group.

Apparatus

The animals were trained to drink from two 100 ml plastic graduated cylinders fitted with rubber stoppers and stainless steel drinking spouts clipped to the front of the home cages. Separate drinking tubes were used for saccharin solution and water. Saccharin solution was prepared every 3 days in distilled water, sodium saccharin 0.125% (w/v), kept at room temperature and stored in a room adjacent to the animal lab. Animals were exposed to the saccharin solution only at designated times during the experiment to avoid possible interference of olfactory cues in the acquisition and extinction of taste aversion [1].

Procedure

The animals were randomly divided into six equal groups designated as: SASA (physiological saline, 0.9%, treatment on Days 1 and 2), LISA (LiCl treatment on Day 1 and saline treatment on Day 2), SAPH (saline treatment on Day 1 and phenobarbital treatment on Day 2), LIPH (LiCl treatment on Day 1 and phenobarbital on Day 2), SABA (saline treatment on Day 1 and barbital treatment on Day 2) and LIBA (LiCl treatment on Day 1 and barbital treatment on Day 2).

All animals were adapted to a 23 hr fluid deprivation schedule for 7 days. On Day 1 of the experiment all the rats were offered 0.125% saccharin solution in both tubes for the 1 hr drinking session. Thirty minutes after drinking 18 rats (the LiCl groups) were injected SC with 3.0 mEq/kg LiCl dissolved in distilled water and administered in a volume of 4.61 ml/kg. This dose of LiCl given SC has been reported to be the most effective in the induction of conditioned taste aversion [6]. The remaining 18 animals were injected SC with 4.61 ml/kg of 0.9% saline.

On Day 2, three groups of 12 animals each were injected SC with either 0.9% saline (SASA and LISA groups), 60 mg/kg sodium phenobarbital (SAPH and LIPH groups) or 80 mg/kg sodium barbital (SABA and LIBA groups) 15 min before the 1 hr drinking session. Barbiturates were dissolved in 0.9% saline and administered in a volume of 1 ml/kg. Discrimination testing was initiated in the following manner. The animals were removed from their cages and two tubes containing either saccharin or water were clipped randomly to either side of the front of each cage. The animals were then returned to their home cages facing away from the drinking tubes towards the back of each cage. The position of the tubes was reversed after 30 min during the drinking session.

For the next 18 consecutive days both saccharin and water were available for the 1 hr drinking session. The tubes were always clipped to the cages while the animals were being weighed. The starting position of the tubes on each day was determined by tossing a coin and the position of the tubes was always reversed 30 min into the drinking session. All drinking fluids were kept at room temperature. Food was available except on Days 1 and 2 to avoid possible food aversion effects. The amount of saccharin or water consumed for the 30 min periods for each rat was recorded in milliliters. Percent aversion scores for each animal on each day was calculated by the following formula:

$$PA=[\text{Water intake (ml)}/\text{Total fluid intake (ml)}]\times 100$$

The arc sin square root transformation was determined for each data point. Data were analyzed by a three factor ANOVA with repeated measures. The factors were Day 1 treatment, 2 levels consisting of saline or LiCl administration, Day 2 treatment, 3 levels consisting of saline, phenobarbital or barbital administration, and Days, 20 levels with repeated measures consisting of 20 days of aversion testing. Simple main effects and Tukey A tests [13] were used for analysis on the data obtained on Days 2, 3, 5, 10, 15, and 20.

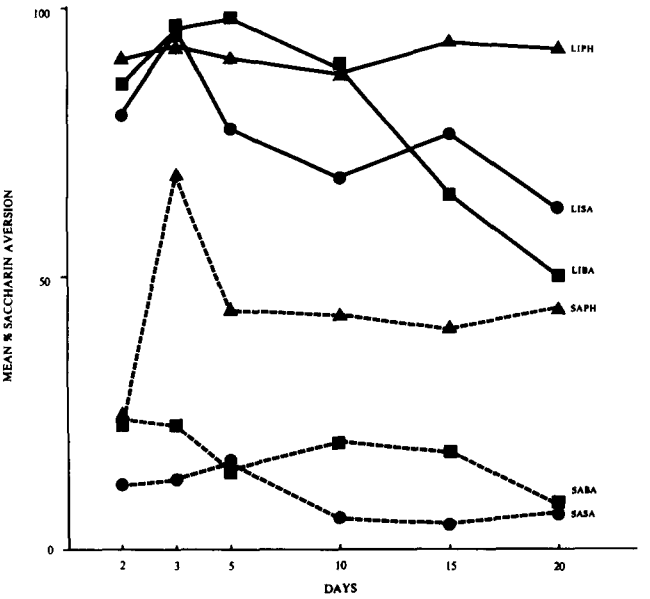

FIG. 1. Mean percent aversion scores obtained for all groups on Days 2, 3, 5, 10, 15, and 20. Barbiturates, 60 mg/kg phenobarbital (▲) and 80 mg/kg barbital (■), or saline (●), were administered on Day 2 fifteen minutes before the drinking session. LiCl treatment on Day 1 (solid lines) and 0.9% saline treatment on Day 1 (dotted lines).

RESULTS

Mean percent aversion scores for each group on Days 2, 3; 5, 10, 15, and 20 are shown in Fig. 1. Data for other days are omitted from the figure for clarity. The overall main effects for treatment on Day 1 (LiCl or saline), $F(1,30)=127.69$, $p<0.01$, and Day 2 (saline, phenobarbital or barbital), $F(2,30)=8.04$, $p<0.01$, were significant. The overall effect for Days (1–20) was also significant, $F(19,570)=23.62$, $p<0.01$. Only the Day 1 and Day 2 Treatment×Days interaction terms were significant, $F(19,570)=6.82$, $p<0.01$; $F(38,570)=2.01$, $p<0.01$, respectively. The three-way interaction term, Day 1×Day 2×Days, was also significant, $F(38,570)=1.63$, $p<0.01$. Simple main effects analysis comparing all groups at each selected day revealed significant differences, at Day 2, $F(5,570)=18.482$, Day 3, $F(5,570)=26.958$, Day 5, $F(5,570)=24.815$, Day 10, $F(5,570)=23.393$, Day 15, $F(5,570)=18.701$, and Day 20, $F(5,570)=22.779$, $p<0.01$. Post hoc Tukey A tests revealed that on Day 2 the 3 LiCl injected groups displayed greater saccharin aversion than the 3 saline injected groups ($p<0.01$), while on Day 3 the SAPH group displayed greater saccharin aversion than the other two saline groups ($p<0.01$), which was not different from that displayed by the LIPH group. On Day 5, again the three LiCl treated groups exhibited significantly more aversion to saccharin than the

saline treated controls ($p<0.01$). However, the comparison between the LISA and the SAPH groups was not significant. Therefore, also on Day 5 the level of saccharin aversion induced in the SAPH group is elevated over that observed in the SABA group but not different than that observed in the LISA group. Note that on this day the levels of aversion produced by LiCl and saline, LiCl and phenobarbital and LiCl and barbital are not different. On Day 10, the magnitude of saccharin aversion in the SAPH group was significantly ($p<0.01$) elevated over that produced by barbital (SABA) and saline (SASA), while the saccharin aversions of the LISA, LIPH, and LIBA groups were not significantly different from one another. Again, the SAPH group aversion was not different from that of the LISA group. On Day 15 the saccharin aversion exhibited by the SAPH group was significantly greater than the SABA group, $p<0.01$, while the three LiCl groups; LISA, LIPH, and LIBA, exhibited significantly more saccharin aversion than the SASA group and the SABA group, $p<0.01$, but were not different from the SAPH group. Tukey A tests on Day 20 revealed that the LIPH group was significantly different from all other groups, $p<0.01$; whereas, the SAPH, LISA and LIBA groups were not different from one another but were elevated in comparison to the SABA and SASA groups, $p<0.01$.

DISCUSSION

The present results illustrate two points. First, phenobarbital is a more effective taste aversive inducing agent than barbital. Second, phenobarbital when given subsequent to and in combination with LiCl produces a relatively strong saccharin taste aversion of longer duration than that produced by either LiCl plus barbital or LiCl alone. These data also indicate that the attenuation effect observed for both barbiturates when administered 72 hr after LiCl [3] might be a phenomenon specific to the periodic forced saccharin exposure.

Saccharin intakes observed for the SAPH group were variable. Some animals displayed a severe saccharin avoidance while others did not. This accounts for the overall 50% aversion scores obtained for this group. The obvious decrease in saccharin intake on Day 3 by the SAPH group might be attributed to differences in half life between phenobarbital and barbital. It should be noted that in this model the drug was administered 24 hr prior to drinking the saccharin solution of Day 3. Whereas, on Day 2 animals were permitted to drink the saccharin 15 min after the drug was administered and this pronounced decrease in intake did not occur. Animals in the LIPH groups drank very little saccharin (0–5 ml) throughout the experiment while some animals in the other two LiCl groups sometimes drank up to 10 ml. This level of saccharin intake varied and more animals tended to drink 5–10 ml saccharin towards the end of the experiment. In general, water intake tended to increase over the duration of the experiment. Such an effect might confound the interpretation of the results because an increase in water intake with no accompanying saccharin increase would produce an increase in aversion scores. However, since water intakes generally increased for all groups, the observed saccharin aversion by the LIPH groups cannot be solely attributed to this effect.

Body weight did not vary throughout the experiment although a decrease was observed in all groups following initial fluid deprivation and in the LiCl treated animals after Day 1. However, body weight in all groups recovered and remained stable throughout the duration of the experiment.

Although the present model is not directly comparable to that employed by other workers [3, 4, 10], these results can be utilized to explain the observed post-barbiturate effects on saccharin consumption. A relatively permanent and maximum effect occurred with both drugs for the first 10 days. The effect persists with phenobarbital for at least the next 10 days; whereas the taste aversion in the LiCl and barbital group extinguishes significantly. Phenobarbital alone also produced a more severe taste aversion than did barbital. Thus, the post-drug decrease during periodic forced extinction, 72 hr after LiCl, is probably the result of a facilitated barbiturate induced saccharin aversion by LiCl. Since pentobarbital and secobarbital also induce differential effects on LiCl taste aversion [4,10], it would have been interesting to have tested these drugs under the present conditions.

The fact that phenobarbital alone (SAPH) produces a relatively long enduring taste aversion which is significantly less than the synergistic effect of LiCl plus phenobarbital and the fact that the aversion due to LiCl begins to extinguish after 10 days (LISA) indicates that the long term effect in the LIPH group can be attributed to the phenobarbital and the increased magnitude of the effect is due to a facilitation by the LiCl. The long duration of the aversion effect might be due to serum protein binding of the phenobarbital and the well known slow renal excretion of some barbiturates. The increased magnitude of the aversion effect might be attributed to some specific co-transport substitution between sodium and lithium and the possibility that phenobarbital is sequestered in some other compartment such as the cerebrospinal fluid. Serum, cerebrospinal fluid, and urine determinations of phenobarbital under these conditions are required before a complete interpretation of the present results will be possible.

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