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Change in Atropine Dose Effect Curve After Subacute Soman Administration[1]

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MODROW, H E AND J H McDONOUGH *Change in atropine dose effect curve after subacute soman administration* PHARMACOL BIOCHEM BEHAV 24(4) 845–848, 1986 —Thirty-one adult male rats were trained on a two component (FR10-extinction) operant task After establishment of an atropine sulfate dose effect curve (vehicle, 1 0, 1 8, 3 2, 5 6 or 10 0 mg/kg), all rats were injected SC with 35 0 μg/kg soman three times per week for four weeks One, twenty-eight and fifty-six days after the last soman injection, additional atropine tests were given Atropine produced significant dose related decreases in FR10 responding at 3 2, 5.6 and 10.0 mg/kg during the initial dose effect curve with no effect on extinction responding. Although four rats died during the soman exposure period, the remaining rats exhibited neither signs of organophosphate toxicity nor significant disruptions in FR responding However, a significant increase in extinction component responding was seen on soman injection days The atropine dose effect curve for FR10 responding showed a significant shift to the left on the day after the last dose of soman However, the dose effect curves at 4 and 8 weeks were the same as the initial curve These results provide behavioral evidence that chronic soman exposure results in a supersensitivity to the antimuscarinic drug, atropine, possibly due to a down-regulation in the number of muscarinic receptors

Atropine sulfate Organophosphate Soman Supersensitivity operant behavior Behavioral tolerance

THE organophosphate compound soman (pinacolyl methylphosphono-fluoridate) is a potent irreversible cholinesterase inhibitor, exerting significant effects on both learned and unlearned behaviors [9, 10, 11, 14, 23] Although a single injection of soman produces both dose related reductions in operant responding [11] and acute reductions in cholinesterase (ChE) activity [20], repeated organophosphate administrations produce rapid behavioral tolerance with a return to pre-injection levels of responding [1, 2, 13, 15, 16, 17, 18] despite continued reduced levels of ChE activity. After administration of paraoxon every third day for forty days, avoidance performance in rats was reported to be within normal limits despite ChE levels of less than 10% of normal [2]. Repeated administration of DFP lowered ChE levels to approximately 20% of pre-injection levels and produced a slight depression in extinction responding [7]. After cessation of the DFP administration, ChE levels returned towards normal and extinction responding increased beyond that of control levels [7]. This might indicate development of a behavioral tolerance during ChE inhibition and overcompensation as ChE activity returns to normal.

Toxicological studies of soman by Sterri and colleagues [21,22] in mice, rats and guinea pigs have shown that repeated administration of 0.5 LD50 doses of soman every 24 hr for 7 to 12 days results in a subpopulation of animals that are able to survive a total cumulative exposure 4 to 6 times the acute LD50. This effect appeared to be species dependent, guinea pigs being more resistant than either rats or mice. These investigators have attributed the observed effect to distribution factors since blood plasma pseudocholinesterase and aliesterases, both of which bind soman, recover to 70 and 100% respectively of their normal activity levels within 24 hr after dosing, thus serving as a sink for the soman during repeated exposures Alternatively, it has been recently reported that subacute administration of soman produces a decrease in the number of muscarinic receptors in the rat brain [3]. The decrease in receptors was specific to cholinergic nuclei (hippocampus, pyriform cortex, etc.) and receptor numbers were found to return to normal levels within 24 days after the last soman injection. Overstreet *et al* [12] previously hypothesized that a decrease in muscarinic receptors may be the basis for behavioral tolerance. If an actual decrease in the number of central muscarinic receptors was to occur, a behavioral supersensitivity towards an antimuscarinic drug should be observed as the same amount of drug would act at a higher percentage of all possible receptors thus producing an increased reaction. Although after repeated exposure to DFP, rats develop a supersensitivity to the stereotypic behavior elicited by high doses of atropine [19], there have been no reports that repeated exposure to soman results in the development of behavioral tolerance The reason for this may be that the

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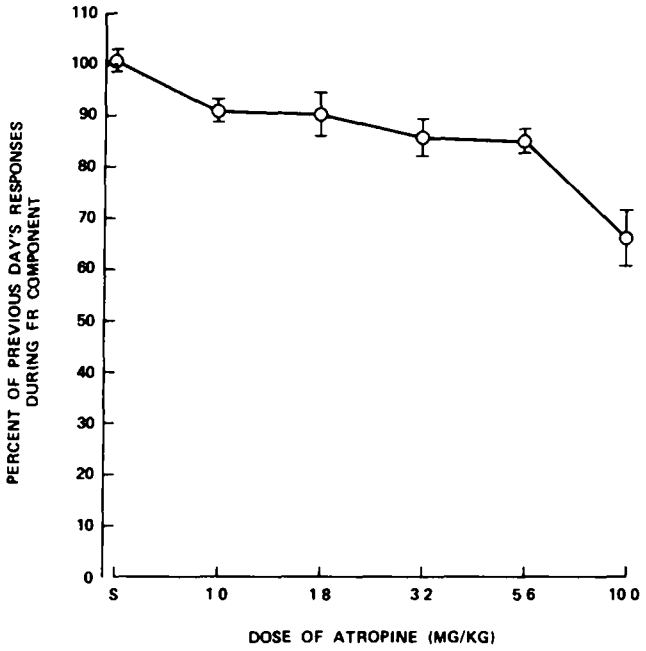

FIG 1 The significant dose-related decrease in FR responding due to injection of atropine Each rat (n=31) was tested at every dose using a Latin Square design The figure shows percent of the previous day's response rate ±S E M

majority of behavioral research has shown that soman exerts its behavioral effects only at doses >0 5 LD50, leaving only a narrow margin between behavioral and toxicological effects [10, 11, 14].

The purpose of the present study was to determine whether subacute exposure of rats to the organophosphate compound soman would produce a supersensitivity to effects of the antimuscarinic compound atropine on an operant task If supersensitivity due to the decrease in muscarinic receptors does occur, then it would be predicted that the same dose of an antimuscarinic compound, atropine, would produce differential effects when given before and immediately after long term exposure to soman. As the number of muscarinic receptors returned to normal levels after cessation of soman exposure, the behavioral effects of atropine would also return to pre-soman levels

METHOD

Subjects

Thirty-one adult male, Sprague-Dawley rats (Charles River), weighing between 275 and 350 grams, served as subjects They were individually housed in stainless steel wire rack cages with ad lib access to water. Each subject was maintained at approximately 85% of its free-feeding body weight food, using a restricted feeding schedule The daily food ration was given to animals approximately 2 hr after the experimental session each day The animals were maintained on a 12 hr 12 hr light dark cycle with light onset at 0600 each day in a temperature and humidity controlled room

Apparatus

The equipment used in this study consisted of four identical BRS-LVE operant conditioning chambers. All chambers were located within individual sound attenuating cubicles equipped with a ventilation fan Each operant chamber (26 7×30 5×24 1 cm) was equipped with a house light, a single response lever and a dipper mechanism for delivery of 0 01 ml milk reinforcement (Eagle brand sweetened condensed milk diluted 1 2 v v with water) All response and reinforcement parameters were controlled by means of solid-state programming and electromechanical equipment located in a separate room

Procedure

Each animal was initially trained by the method of successive approximation to lever press for milk reinforcement on a fixed ratio 1 (FR1) schedule Over the next five to ten sessions the response requirement was gradually raised to FR10 during 30 minute daily sessions At this point, the operant schedule was changed to a two component operant task The first 20 min of each session continued to consist of the FR10 schedule for milk reinforcement while during the last 10 min period, the rats were on an extinction schedule, i e , lever presses would not result in delivery of reinforcement There were no changes in environmental stimuli to indicate the onset of the extinction component. All training and test sessions were 30 min long with sessions conducted five days per week Following attainment of a stability criterion (no more than a 10% deviation from the mean for both FR and extinction responses over three consecutive days), a dose effect curve for atropine sulfate was obtained for all rats Using a Latin Square design, rats were tested 30 min after IM injection of vehicle, 1.0, 1 8, 3 2, 5 6, or 10 0 mg/kg atropine Test sessions were conducted identically to training sessions with the exception that tests were only conducted on Tuesday through Thursday Additionally, tests were given no more than once per week and animals were required to reattain the stability criterion prior to each test

One week after completion of the atropine dose effect curve, each animal was randomly assigned to one of four test groups All rats then began a series of subacute soman injections During this series, all animals were injected SC with 35 μg/kg soman 3 times/week (M, W, F) for 4 weeks and given training sessions on the two component (FR-extinction) task Equivalent volumes of saline were injected SC twice a week (Tu, Th) prior to the training session Operant sessions were conducted 30 minutes after all injections The day after the last soman injection, each animal was injected IM with one of four previously chosen doses of atropine sulfate (1 8 mg/kg [n=6], 3 2 mg/kg [n=7], 5 6 mg/kg [n=7], or 10 0 mg/kg [n=7]) depending upon group assignment Each animal was also tested with the same dose of atropine four and eight weeks after the completion of the subacute soman dosing schedule During the time between atropine tests, post-soman exposure training sessions continued five days per week

Drugs

All drug doses are expressed as salts Atropine sulfate (Boehringer, Lot No 352) was dissolved to appropriate concentrations to deliver 1 ml per kg of body weight Soman (97% pure) was prepared in saline to a concentration of 35 μg/ml Atropine solutions were stored under refrigeration between tests while the soman solutions were prepared fresh for each injection day

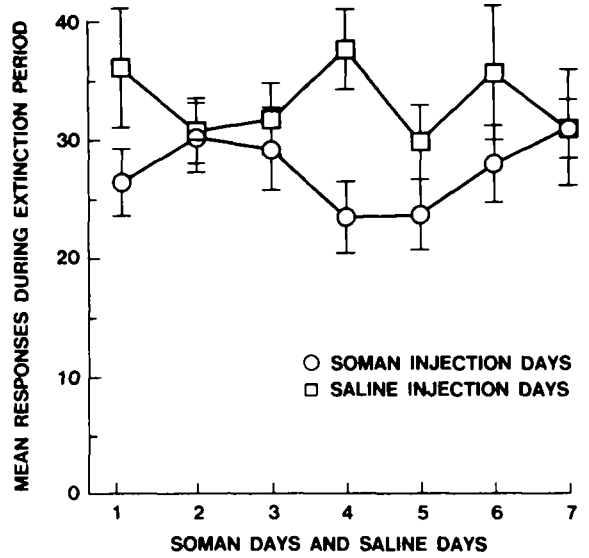

FIG 2 The significant difference in extinction component responding during soman injection days when compared to saline injection days Lines indicate S E M There was no significant change in response rate from the first injection of either soman or saline to the last injection

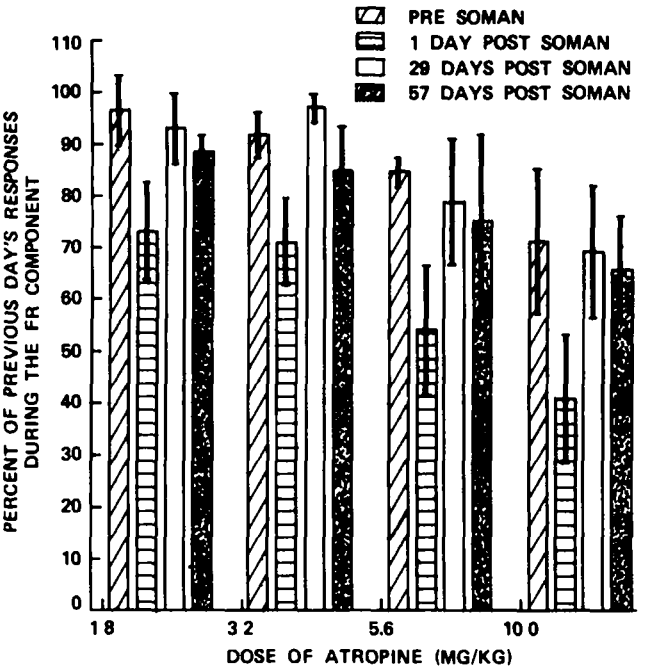

FIG 3 The significant decrease in FR component response rates on the day following the final soman injection with a return to pre-soman levels within 29 days after cessation of soman injections Lines indicate S E M Rats were randomly assigned to one of the four dose groups (1 8 mg/kg n=6, 3 2 mg/kg n=7, 5 6 mg/kg n=7, 10 0 mg/kg n=7)

RESULTS

Prior to the repeated soman injections, atropine sulfate produced a significant dose dependent decrease in responding during the FR10 component, $F(5,150)=15.44, p<0.01$. As shown in Fig. 1, doses of 3.2, 5.6 and 10.0 mg/kg atropine lowered responding significantly below that produced by the vehicle injection (Tukey post-hoc analysis, $p<0.05$) (Kirk, 1968), with 10.0 mg/kg producing a response rate significantly less than that observed at any other dose (Tukey, $p<0.01$) A comparison of responding during the first and second 10 minute portions of the 20 minute FR component also revealed a significant difference in response rates between the two time periods, $F(1,30)=19\ 86, p<0.01$, with no significant interaction found between drug dose and FR period. Thus, the conclusion can be made that atropine does not affect the "warmup" effect seen at the beginning of each session. Despite significant drug effects during the FR component, there was no significant drug effect during the extinction period, although there was a trend towards elevated response rates That is, although a significant dose related drug effect was seen during the reinforced component, during the extinction period of the same session, no reliable differences were seen between any atropine doses.

Four rats died during the subacute soman exposure period Surprisingly, the remaining animals exhibited no significant change in FR response rate during this period. Additionally, there was no significant difference in response rate between the soman injection days and the saline injection days. The lack of significant change in response rate was found for only the FR component of the schedule. The extinction component displayed a significant difference between the number of responses shown on soman injection days and the following saline injection day, $F(1,27)=7.52$, $p<0.05$ However, there was no significant change from the first injection to the final injection for either saline or soman injections (Fig 2). Only when all saline injection days were compared to all soman injection days was a significant difference found.

When the animals were given atropine one day after the end of the subacute soman injections, a significant disruption in FR responding was seen, $F(3,9)=6\ 16, p<0.01$. The disruption of FR performance by atropine was of a greater magnitude than that seen either before subacute soman administration or in the tests four and eight weeks after subacute soman administration (Tukey post-hoc analysis, $p<0.05$). These data are presented in Fig. 3. Tests given four and eight weeks later produced responding not significantly different from that produced by the same animals prior to the subacute soman (Tukey post-hoc analysis). This figure also demonstrates that all tests produced similar dose-dependent reductions in FR responding, $F(3,23)=6\ 06, p<0\ 01$, the only difference being the significantly greater reduction produced by all doses of atropine on the first day after soman. Additional Tukey post-hoc analyses determined that 5.6 and 10.0 mg/kg atropine produced lower response rates than the 1 8 mg/kg dose at all test times. Therefore, although the subacute administration of soman did not by itself produce a significant change in behavior, when the rats were challenged by the muscarinic antagonist atropine sulfate 24 hr after the final soman injection, a significant change in behavior was seen

DISCUSSION

The effects of atropine injections confirm the results of previous studies [8]. Atropine produced a significant dose-related decrease in responding on a FR10 schedule. Injections of 3.2, 5.6 and 10.0 mg/kg of atropine produced a re-

sponse rate significantly below control levels during the FR component This is not surprising since atropine has previously been shown to possess rate decreasing effects [8] A surprising result was the lack of effect observed during the extinction period. Higher doses of atropine might be expected to produce a disinhibitory effect on extinction responding. However, no dose of atropine significantly increased responding during the extinction period

Subacute administration of soman did not significantly disrupt performance during the FR component. Even the first injection of soman did not significantly decrease the mean response rate during this component Other investigators have found a significant disruption after a single injection of an organophosphate [2, 4, 5, 6] Although there were no changes during the FR component, a significant difference between the soman and saline days in the number of responses during the extinction component of the task was seen The changes during the extinction component results directly confirm the observations of Glow and Rose [7] Two hypotheses may explain these effects. The first possibility is that an overlearned response such as this is more resistant to disruption by an organophosphate This hypothesis would also state that performance on a previously learned task might be less affected than learning a new task after chronic administration of organophosphates [1] The other possibility is that the dose utilized in this study was physiologically less potent than those utilized in previous studies so that it did not produce rapid decreases in ChE levels Rather, the ChE levels may have been gradually reduced thereby allowing more rapid acquisition of behavioral tolerance

The most important finding of this study was that soman administration did cause previously tested doses of atropine to produce significantly greater decrements in responding immediately after termination of soman administration. Russell *et al* [15, 16] also found an increased sensitivity to the rate-decreasing effects of atropine after DFP injections However, the finding that the atropine dose-effect curve returned to pre-soman administration levels within 29 days after termination of soman is unique These results suggest that the behavioral time course may be very similar to the time course of recovery of muscarinic receptors within the central nervous system [3] Therefore the conclusion can be made that although behavioral tolerance rapidly develops to the reduced number of muscarinic receptors produced by subacute administration of soman, the behavioral tolerance is also accompanied by a supersensitivity to muscarinic compounds. When the soman administration ends, the decline in behavioral supersensitivity is paralleled by the concomitant increase in muscarinic receptors [3]

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