Pharmacology Biochemistry & Behavior, Vol 4, pp. 725–728.

BRIEF COMMUNICATION

Time Dependent Action of Pimozide on Deprivation-Induced Water Intake: Evidence for a Direct Drug Effect[1]

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(Received 9 February 1976)

GRUPP, L. A *Time dependent action of pimozide on deprivation-induced water intake evidence for a direct effect* PHARMAC. BIOCHEM. BEHAV. 4(6) 725–728, 1976 – Time-response curves were established for the effect of 3 doses of pimozide, a potent and specific dopamine receptor blocker, on deprivation-induced drinking in rats. Injections occurred once every 3 days at intervals of 2, 4, 8, and 16 hr prior to the daily 15 min drinking period. Pimozide produced a significant attenuation in the amount of water normally consumed (on nondrug days) whose degree varied with the proximity of the injection to the drinking period Only the 2 higher doses (0.5 and 1 0 mg/kg) produced a significant dose dependent effect which was strongest at the 2 hr interval and which gradually weakened by the 8th post injection hr The relevance of these findings for a direct versus an indirect action of the drug on water intake is discussed.

Pimozide Deprivation-induced drinking Dopamine receptor blocker

PIMOZIDE, a long acting neuroleptic of the diphenylbutylpiperdine series [4], has been shown to have an influence upon a number of learned (e.g., avoidance responding [2,4], amphetamine self administration [16], self stimulation [8,11]) and unlearned behaviours (e.g., hypothermia [6], hyperkinesia [13], amphetamine and apomorphine induced stereotypy [4, 5, 12]). Since this agent is thought to have a specific blocking action on central dopaminergic receptors [1], the above results have been taken to demonstrate that neurochemically, all these behaviours may be in part mediated via central dopaminergic mechanisms.

Recently, a number of investigators have demonstrated that pimozide is also capable of producing modifications in the regulation of water intake. Both Zis and Fibiger [17] and Nielsen and Lyon [10] reported that doses ranging from 0.45–1.0 mg/kg were capable of producing a significant reduction in the water intake of deprived rats, and that this reduction could not be accounted for simply in terms of a drug induced motor deficit. Since a number of different neurotransmitters can affect fluid consumption [3, 9, 14] (pimozide itself is capable of decreasing striatal acetylcholine levels [7]), it is of interest to determine whether pimozide produces its effects on water intake directly (presumably via dopaminergic blockade) or indirectly through an active metabolite of the parent substance. It is known that the pimozide content in the brain reaches its maximum shortly after injection (1 hr) and begins to decline thereafter, whereas the level of pimozide metabolites remain constant in the brain for approximately 7 hr following the injection [4]. If pimozide's depressing effect on water consumption were due to a direct action of the drug, one might expect a given dose to produce its maximal effect on drinking at a time when it was maximally concentrated in the brain, i.e., by at least the first postinjection hr, and to decline in effect thereafter. However, if the decrease in water intake were due to an indirect effect of the drug, (e.g., an active metabolite) one might expect a given dose to produce a maximal and relatively constant effect for a 7 hr period subsequent to the injection and then to decline in effect. The following experiment attempts to answer this question by tracing the drug's effect on water consumption up to sixteen hours post-injection.

METHOD

Animals

The animals were 20 drug naive male hooded rats weighing 370–500 g at the beginning of the experiment. All animals were individually housed and given ad lib access

[1] ORAP^{R} (pimozide) – A generous supply of pimozide was provided as a gift from Dr W J. Forgiel of McNeil Laboratories, Don Mills, Ontario.

to food and water A 12 hour light-dark cycle was in effect throughout.

Procedure

To begin the experiment water bottles were removed from the home cages and access to water was restricted to a 15 min drinking period given at 24 hr intervals. During this period the animals were removed from their home cages and placed in a drinking cage which differed from the home cage only in that a 100 ml Richter tube was attached to the front. No food was available in the drinking cage As a matter of routine all animals were weighed daily immediately prior to the drinking period.

When the animals were completely adapted to this procedure and the amount of water consumed had stabilized (this occurred by the sixth day of the restricted water regime), the animals were randomly divided into 4 groups of 5 animals each and each group was assigned to 1 of the following 4 drug treatments Group P_1 (0.25 mg/kg pimozide), Group P_2 (0.5 mg/kg pimozide), Group P_3 (1 0 mg/kg pimozide) and Group Veh (acetic acid-5% glucose solution). Statistical analysis revealed no significant differences between any of the 4 groups in the amount of water consumed on the final 2 days of the restricted water regime (2-tailed t test $p<0.05$). The drug and vehicle were administered by IP injection and concentrations were adjusted so as to give 1 ml of fluid per 100 g of body weight Pimozide was suspended in a 5% acetic acid-glucose solution.

Schedule of Injections. The 4 treatment groups were tested with their respective doses of pimozide or vehicle only once every 3 days (test day) so that a cycle of 1 test day followed by 2 normal (i.e., nondrug) days followed by a test day etc was in effect. Using this basic schedule of drug administration, the differential effect on water consumption of 4 different time intervals between injection and the drinking period was examined The intervals chosen and their order to testing was 2 hr, 16 hr, 8 hr, 4 hr, 2 hr, and a block consisting of 5 test days and 10 nondrug days was devoted to each time interval. In order to facilitate the scheduling of injections all 4 treatment groups were tested simultaneously at the same time interval before moving on to the next The 2 hr interval was repeated at the end of the experiment so that comparison with the results of the initial 2 hr interval block of tests would allow the assessment of possible tolerance effects. Thus all animals were subjected to 5 test days at each of the 5 time intervals to yield a total of 25 test days per animal, and each treatment group as a unit accumulated a total of 25 test days at each time interval.

RESULTS

An initial analysis of variance revealed no significant differences in water consumption between the first and last sets of 2 hr interval tests This indicated that at least on this measure, no tolerance to pimozide's effect developed over time and thus allowed a further analysis of variance to be done with the values of these two 2 hr interval tests combined This analysis was carried out on the scores obtained by subtracting the amount drunk on a test day from the amount drunk on the preceding water day and revealed a significant effect of drug dose ($F(3,12) = 31.6$, $p<0.0005$) a significant interval effect ($F(3,472) = 21.0$, $p<0.0005$) and a significant interaction between dose and interval ($F(9,472) = 4.83$, $p<0\ 0005$). Thus pimozide brought about a significant decrease in water consumption whose magnitude varied with dose and with the interval between administration and access to water.

Figure 1 illustrates the time-response relationship for water consumption on test days for all 4 groups These data points were derived by expressing for each animal the mean amount drunk over the 5 test days at a given interval as a percentage of the mean amount drunk over the 5 immediately preceding nondrug days and then calculating the average for all animals in a given drug group A series of 1-tailed t tests (with the criterion for significance set at the 0.05 level) performed on the data revealed the following findings for the effect of dose and interval

Dose

Across all intervals tested the P_1 group did not differ significantly from the Veh group. The 0 25 mg/kg dose of pimozide used in the P_1 group therefore, had no effect on water consumption and for this reason will not be considered in any further detail in this section The P_2 group drank significantly less water than both the Veh and P_1 groups at the 2 hr interval but not at the 4, 8, and 16 hr intervals. Finally the P_3 group drank significantly less water than both the Veh and P_1 groups at the 2, 4, and 8 hr intervals and significantly less than the P_2 group at the 2 and 4 hr intervals only There were no significant differences between any of the 4 groups at the 16 hr interval Thus the tendency was for the larger doses of pimozide to produce the stronger effects on water consumption across all the intervals tested

Interval

Within the Veh group no significant differences in water consumption emerged at any of the intervals indicating a neutral effect of the vehicle on water consumption. Within the P_2 group water consumption at the 2 hr interval was significantly less than at the 4, 8, and 16 hr intervals, while consumption at the 4 hr interval was significantly less than at 8 but not at the 16 hr interval The effect of interval in the P_3 group exactly paralleled that of the P_2 group with the exception that consumption at the 4 hr interval was significantly less than at the 16 hr interval Finally, in all of the groups no significant differences in consumption were seen between the 8 and 16 hr intervals. Thus for both P_2 and P_3 groups, the effect on water consumption decreased steadily with time and eventually disappeared by the 8th postinjection hr

DISCUSSION

The results of this experiment confirm earlier reports [10, 15, 17] that pimozide, a specific dopaminergic receptor blocker, is an agent capable of producing deficits in deprivation-induced water intake. In addition our results show that this effect is a graded one with a maximum effect occurring at least 2 hr postinjection and then decreasing in strength up to the 8th postinjection hr after which no effect on water intake is observed. Only the 2 higher doses of pimozide used (0.5 and 1.0 mg/kg) had any significant effect on water intake at the intervals tested, while the lowest dose (0 25 mg/kg) did not differ significantly from the Vehicle control group. This result is in agreement with that of other investigators [10] who showed that doses of pimozide at 0.5 mg/kg or greater were capable of decreasing water consumption.

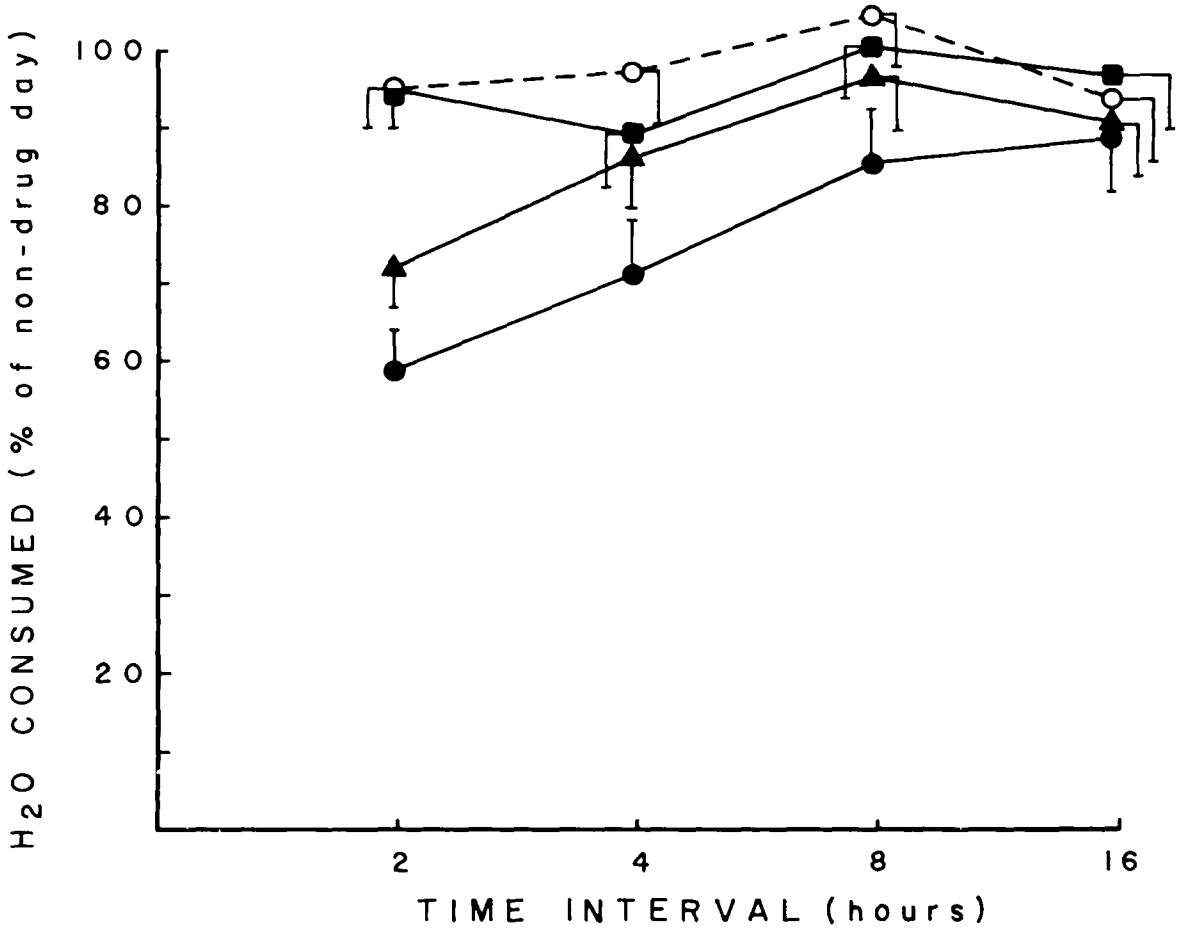

FIG. 1. Amount of water consumed for each treatment group across the 4 time intervals tested. The data are expressed as a percentage of the amount drunk on the immediately preceding nondrug day, each point being the mean for the 5 animals in each treatment group The abscissca plots the time interval on a logarthmic scale and the vertical bars give the within subject confidence intervals Group P_1 is indicated by ■——■, Group P_2 by ▲——▲, Group P_3 by ●——●; Group Veh by ○– – –○.

Janssen *et al.* [4] in their very detailed study of the kinetics of pimozide showed that the tritiated material content of the brain (i.e., pimozide plus metabolites) reaches a maximum level at the first hr postinjection and persists, practically unchanged, at this level for a further 7 hr after which it declines On the other hand, pimozide content in the brain was found to be highest 1 hr after injection and to decrease thereafter until by the 8th hr postinjection it attained only about one third of its 1 hr level These findings, when applied to the present data, suggest that the effect over time of pimozide on water intake is paralleled more closely by the function describing pimozide content in the brain than by that describing pimozide metabolite content in the brain. Thus over a 16 hr postinjection period, the ability of a particular dose of pimozide to produce a decrease in water intake dissipated rather than remained constant. Taken together these data suggest that pimozide produces its effect on water consumption directly and most probably through dopamine receptor blockade, rather than by the action of an active metabolite. It should be pointed out that in Janssen's experiments pimozide was administered subcutaneously, while in the present experiment the intraperitoneal route was used. This difference in route of administration would not be expected to affect the general shape of the curves obtained (i.e., the time course of the effect) but rather the point in time at which the drug effect begins. The present data do not necessarily demonstrate that pimozide produced the decrease in intake only by affecting central dopaminergic thirst mechanisms, for it is possible that the decrease is also due, in part, to a deficit in motor activity which would reduce the animal's ability to perform the consummatory response [4,11]. On the other hand, it is interesting to note that the 0 25 mg/kg dose of pimozide which has been shown to be effective in antagonizing stereotyped behaviour (i e., reducing motor activity) failed to produce a significant decrease in water intake in the present study. Thus to the extent that the effect on water intake is not entirely attributable to a motor deficit these findings lend further support to the notion that dopaminergic activity is involved in the control of water intake.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. B. Thomas for his capable technical assistance, Dr. Howard Kaplan for assistance in the data analysis and Mr. Lonnie Currin for preparation of the graph.

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