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BRIEF COMMUNICATION

Time Course of Pimozide Effects on Brain Stimulation Reward

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ATALAY, J. AND R. A. WISE. *Time course of pimozide effects on brain stimulation reward.* PHARMACOL BIOCHEM BEHAV **18**(4) 655–658, 1983.—Lever pressing for brain stimulation reward (BSR) was tested at hourly intervals following 0.5 or 1.0 mg/kg of pimozide given intraperitoneally. Response suppression was seen within one or two hours and peak effects occurred 5–8 hours after injection. Recovery approached completion within about 24 hours. Since peak brain levels of pimozide are reached quickly, the time course of peak action suggests a complex mechanism of pimozide action.

Pimozide Dopamine receptor blockade Neuroleptics Brain stimulation reward

PIMOZIDE has been used widely in behavioral studies in the rat. The time course of the drug's actions is unusual with respect to other neuroleptics, however [10,14]. With subcutaneous injections pimozide reaches peak concentrations in the brain within one hour after injection [11] but has its peak effects on operant (lever-pressing) behavior between five and eight hours after injection [12]. While such evidence suggests that it is an active metabolite, and not pimozide itself, that has pharmacological action, pimozide displaces haloperidol from dopamine binding sites with normal incubation times [19], and the behavioral actions of pimozide are not delayed by inhibition of oxidative catabolism [14]. It is thus generally assumed that pimozide's behavioral actions result from direct blockade of dopamine receptors [18], and that the delayed action depends on some subtle mechanism perhaps involving a feedback compensation of the dopamine system [14]. It seems clear that the delayed behavioral action of the drug requires careful attention, both in terms of what it reflects regarding the mechanism of action of the drug, and also in terms of more methodological considerations for those who use the drug in behavioral studies.

While the time course of behavioral action of pimozide has been determined only in the case of subcutaneous injections, most behavioral studies have involved intraperitoneal injections. The time between pimozide injections and behavioral testing has varied, usually between two [16, 24, 25], three [4, 15, 31, 32], four [5–8, 20, 22, 27, 28] and five [26] hours, and the time between repeated tests has varied, usually between 24 [1], 48 [7, 10, 25–28] and 72 [4, 5, 20] hours. In the case of subcutaneous injections peak behavioral effects appear to occur between five and eight hours after injection, and recovery approaches completeness within 24 hours. If absorption and time course of action after intraperitoneal injections is the same, it would appear that many behavioral studies have involved testing prior to peak drug effect and are not directly comparable with regard to the effective dose tested, nominal dose equivalence notwithstanding. Moreover, if the intraperitoneal route is the same or significantly slower than the subcutaneous route, some behavioral studies have involved a degree of drug accumulation across repeated drug tests; 24 hours, while sufficient for behavior to return to near-normal levels, is insufficient time for drug clearance between pimozide injections [11]. Because of these possibilities and because the time course of pimozide action is not known in fine detail even following subcutaneous injections, the present study was designed to determine the time-course of pimozide action following intraperitoneal injection in an operant paradigm.

One of the behavioral effects of pimozide and other neuroleptics is impairment of operant behavior motivated by a number of positive rewards including brain stimulation reward [4–8, 12, 13, 15, 24–26, 31, 32], intravenous drug reward [3, 17, 29, 30], and food [20–23, 27, 28] and water [9,10] reward. Of these, brain stimulation reward offers a particularly useful paradigm with which to demonstrate the time-course of neuroleptic action. Neuroleptics cause graded and dose-orderly effects on brain stimulation reward [5, 24, 31], and since there is no deprivation or satiation effect to speak of in this paradigm these effects can be assessed repeatedly in the same animals at varying times after a given injection.

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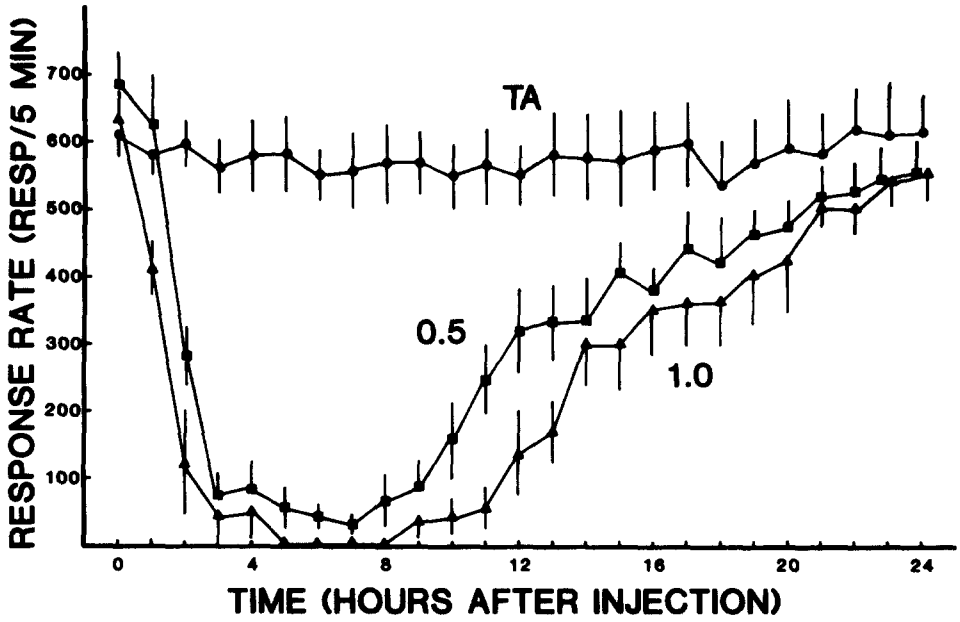

FIG. 1. Response rate (lever-presses per 5 minutes) as a function of pimozide dose and time after injection for a group of 7 rats.

This paradigm was thus used to assess the time course of two doses of pimozide at hourly intervals over a 24 hour period.

METHOD

Seven adult male Long-Evans rats were implanted with monopolar lateral hypothalamic stimulating electrodes. The electrodes were 254 μm stainless steel wires insulated with varnish up to the square cross section of the tip. Stereotaxic coordinates were 0.4 mm posterior to Bregma, 1.7 mm lateral to the midline, and 8.0 mm ventral to the dura; the incisor bar was 5.0 mm above the intra-aural line.

The animals were trained in 26×26 cm operant chambers to lever-press for 0.5 sec trains of sine wave stimulation at 30 μA intensity. They were then given three weeks of daily testing in the self-stimulation paradigm to stabilize their response rates before the first drug test. Six days intervened the three drug tests which were given in orders that were counterbalanced across six of the seven animals.

On drug days testing was done just prior to and hourly for 24 hours after injection. Each hour the animals were given 5 trains of "priming" stimulation followed by a six-minute period in which brain stimulation reward was available. The first minute of this period was treated as a "warmup" period and not scored; response rate was measured over the last five minutes of the test. Stimulation was not available for the intervening periods, but the animals were left in the test box, connected to the inactivated stimulators.

The three drug conditions were 0.5 or 1.0 mg/kg of pimozide or tartaric acid vehicle. Pimozide was dissolved in 0.1 M tartaric acid at a concentration of 0.5 mg/ml, and was given intraperitoneally one hour before the first test period.

Electrode placements were confirmed in 40 μm, thionin-stained, coronal sections.

RESULTS AND DISCUSSION

Response rates are shown as a function of drug dose and time after injection in Fig. 1. Major response attenuation was seen one hour after injection with the high dose and two hours after injection with the low dose. The 0.5 mg/kg dose was less effective in three ways; it had a longer latency, a less severe peak effect, and an earlier recovery time than the 1.0 mg/kg dose.

The present data indicate that pimozide has its mean peak effect at six or seven hours after intraperitoneal injections, and that residual behavioral effects last up to at least 24 hours after such injections. While differences in strength of action between two and three or four hour preinjection times were not statistically reliable in the small sample tested in the present study, the number of points tested allows a reasonably clear estimate of the time course of pimozide action in this preparation, and that time course appears to involve major differences between these preinjection times. These data and those of Janssen *et al.* [12] make it clear that differences in pre-injection time can lead to major differences in behavioral responses to pimozide, and that two, three and four hour pre-injection times cannot be assumed to be equivalent. While shorter pre-injection times are desirable for practical reasons, a six-hour pre-injection time would appear to give the most stable (flattest) effect over a 15 minute to one hour testing period. In studies where shorter preinjection times are used, time as well as dose must be considered an important determinant of strength of effect. Differences in the apparent strength of effect in the range of 3–9 hours after injection may be artificially truncated in this paradigm because of a "floor" effect. With lower doses or in paradigms where less complete behavioral disruptions are seen, it is unlikely that these pre-injection times will have equivalent effects.

The time course of action following intraperitoneal injections fits well with that reported following subcutaneous injections [12]. While the time course following subcutaneous injections was not determined hourly, it showed a peak action somewhere between 5 and 8 hours with almost complete

recovery within 24 hours, as was seen in the present study. Peak brain levels of pimozide are reached within the first hour after subcutaneous injection [11], and similar rapid absorption can be assumed for intraperitoneal injections on the basis of the similar onset and time-course of behavioral action seen in the present study. The present time-effect functions, with their finer detail, would thus seem to be applicable to studies using the subcutaneous route of injection as well, though more comparable testing procedures should be used to confirm this impression. The nearly complete behavioral recovery 24 hours after injection should not be taken to imply that pimozide is completely cleared from the system by this time, however. The drug is still present for two or three days after subcutaneous injection [11], and such periods should be allowed between behavioral tests if drug accumulation is to be avoided.

As other neuroleptics are likely to have different time courses of action [14] and will perhaps have different speeds of absorption and distribution, similar time-course determinations should be done with other agents that are to be compared to pimozide and to each other. Comparisons between neuroleptics are made difficult by the fact that different agents have different side effects, and the need to compare time-effect functions makes such comparisons even more troublesome; however it is clear that such comparisons are necessary and potentially heuristic [14]. The brain stimulation reward paradigm seems particularly well suited for such determinations, since orderly, low-variance data can be obtained in a within-subjects, within-sessions paradigm. Correlation of detailed behavioral time-course data with various neurochemical data at similar post-injection times may shed important new light on the mechanism of neuroleptic action.

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