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The Effects of Chlorimipramine and Protriptyline on Tube Running Activity in Mice

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PERSSON, S.-Å. *The effects of chlorimipramine and protriptyline on tube running activity in mice.* PHARMAC. BIOCHEM. BEHAV. 12(2) 255–258, 1980.—We have developed a new technique, tube running activity, to study the effects of drugs on a specific behaviour in mice. The time it takes for a mouse to run 100 cm in a narrow tube is measured. The effects on tube running activity of chlorimipramine, a relatively specific blocker of the amine pump in the 5-hydroxytryptaminergic neurons, and protriptyline, a relatively specific blocker of the amine pump in the noradrenergic neurons, were studied. During the first two hours after the administration of chlorimipramine 7.5 mg/kg IP there was a decreased run time as compared with controls. The run time could not be further decreased by increasing the dose of chlorimipramine, but the effect was prolonged. Protriptyline in the dose range of 3.75–30 mg/kg IP had no observable effect on the run time, but protriptyline 60 mg/kg IP decreased the run time in the same way as chlorimipramine 60 mg/kg IP did. The decreased run time after protriptyline 60 mg/kg IP is probably due to a blockade of the amine pump in the 5-hydroxytryptamine neurons at this high dose. Our results suggest that tube running activity more specifically measures functional effects of 5-hydroxytryptamine than functional effects of noradrenaline.

Tube running activity Chlorimipramine Protriptyline 5-Hydroxytryptamine Noradrenaline

WE have developed a new technique to study the effects of drugs on a specific behaviour in mice [12,14]. After administration of 5-hydroxytryptophan (5-HTP) mice pretreated with the monoamine oxidase inhibitor nialamide were found to pass through a narrow tube faster than controls. Further experiments involving only monoamine oxidase inhibition and other experiments with the central serotonin receptor activating agent LSD suggested that tube running activity might be a useful tool to quantify functional effects of 5-hydroxytryptamine [13].

The present study was designed to further examine the specificity of the method. Tube running activity was studied after administration of chlorimipramine, a relatively specific inhibitor of neuronal 5-hydroxytryptamine (5-HT) uptake, and after administration of protriptyline, a relatively specific inhibitor of neuronal noradrenalin (NA) uptake [2,3]. The main pharmacological effects of the two drugs are commonly attributed to increased amine levels at the receptor sites leading to an increased receptor activity.

METHOD

Subjects

Male N.M.R.I. mice weighing 20 g were obtained from Anticimex, Sollentuna, Sweden. Batches of 20 mice were kept in makrolon cages (550×330×200 mm). The animals were fed on Anticimex commercial type pelleted diet 213–3. They had access to food and water ad lib., except during the experiments, when only water was available. The artificial light in the animal room was turned on at 08:00 and off at 20:00 hr. The room temperature varied between 22/23°C and the humidity between 35–80%. The mice were allowed to acclimate to these conditions for one week before any experiments. Each animal was used only in one experiment.

Drugs

Chlorimipramine HCl (Ciba-Geigy, Basle) and protriptyline HCl (Merck Sharp and Dohme, Rahway, NJ) were dissolved in 0.9% sodium chloride. The solutions were made just before injection. All drugs were administered intraperitoneally in a volume of 33 ml/kg. If no drug was administered an equal volume of 0.9% sodium chloride was injected. Saline treated mice served as controls.

Procedure

The experimental equipment used in the present experiments has been described elsewhere [14]. It consists of a runway made of a Plexiglas tube with one part of the tube used as a start box. The start box is separated from the rest of the tube by a guillotine door. All experiments were performed between 10:30 and 16:45 hr in a laboratory where the external light was excluded. Ordinary artificial laboratory illumination was used. In order to adapt the animals to the experimental equipment each mouse was placed in a Plexiglas tube of the same dimensions as the tubes of the experimental equipment for the first hour of the experiment. At the time of testing the mouse was put into the start box. The test was started by raising the guillotine door manually. At the same time a stream of air (0.6 l/sec) was started. It blew in

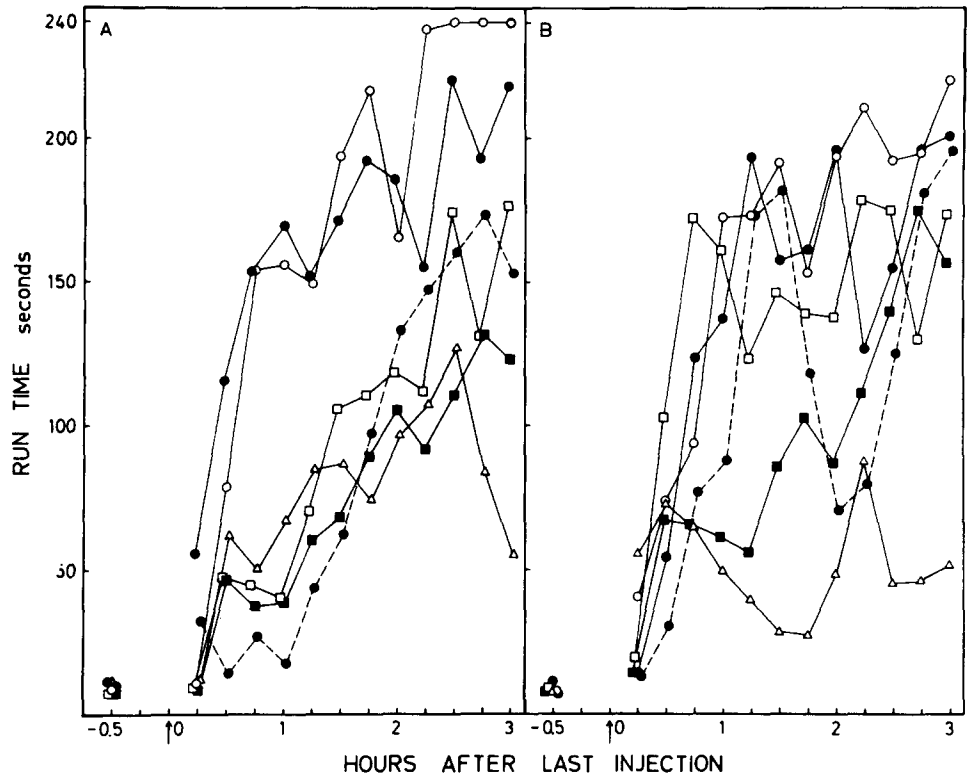

FIG. 1. Tube running activities in mice after IP administration of A. chlorimipramine, B. protryptiline. Each point represents the mean of 7–10 recordings. ○——○ NaCl; ●——● 3.75 mg/kg; ●- - -● 7.5 mg/kg; □——□ 15 mg/kg; ■——■ 30 mg/kg; △——△ 60 mg/kg. The drugs were administered at time 0, as indicated by an arrow.

the direction of the run. The time it took for the mouse to run 100 cm, the run time, was recorded. The maximal run time was set to 240 sec.

Statistical significances were determined with the two-tailed Mann-Whitney U-test.

RESULTS

Mice treated with chlorimipramine, 3.75–60 mg/kg, did not show any observable gross behavioural changes in comparison with the controls. Neither did administration of protriptyline, 3.75–60 mg/kg, cause any changes in the gross behaviour.

Figure 1 A shows the run time of mice treated with various doses of chlorimipramine in comparison with controls. Chlorimipramine, 3.75 mg/kg, had no effect. Increasing the dose to 7.5 mg/kg resulted in a decreased run time, which differed significantly from the run time of control mice with a start first observed 0.5 hr after the administration of the drug (Table 1). A dose of 15 mg/kg had a similar effect but significant differences in comparison with the controls were reached at fewer test points. Chlorimipramine 30 mg/kg did not further decrease the run time, but the effect was prolonged. A similar result was obtained when the dose was increased to 60 mg/kg. Thus, during the first 2 hr after the administration of chlorimipramine 7.5 mg/kg the run time was decreased. No further decrease in run time was observed after increasing the dose of chlorimipramine.

Figure 1 B shows the run time of mice treated with different doses of protriptyline as compared with controls. The run time of mice treated with protriptyline 3.75–15 mg/kg

TABLE 1

P VALUES AT VARIOUS TIMES AFTER THE ADMINISTRATION OF CHLORIMIPRAMINE (Chlo), PROTRIPTYLINE (Pro) AND NaCl

Treatment	Time: hr										
Dose in mg/kg	0.5	0.75	1.00	1.25	1.5	1.75	2.00	2.25	2.5	2.75	3.0
Chlo 7.5 vs NaCl	<0.02	<0.02	<0.002		<0.05	<0.02					
Chlo 15 vs NaCl		<0.02	0.02								
Chlo 30 vs NaCl		<0.02	<0.02		<0.05	<0.05		<0.05	<0.05		<0.05
Chlo 60 vs NaCl		<0.05	<0.05			<0.02				<0.02	<0.002
Pro 7.5 vs NaCl							<0.05				
Pro 15 vs NaCl											
Pro 30 vs NaCl			<0.05	<0.05							
Pro 60 vs NaCl			<0.05		<0.02	<0.05	0.02		<0.05	<0.02	<0.002
Chlo 7.5 vs Pro 7.5			<0.02	0.002	<0.02						
Chlo 15 vs Pro 15		<0.05	<0.02								

Only p values less than 0.05 have been included.

was similar to the run time of the control mice. However, the run time after 7.5 mg/kg differed significantly from the controls at one test point 2 hr after the protriptyline administration (Table 1). After administration of protriptyline, 30 mg/kg, a decrease in run time was observed 1 hr after the drug administration. The run time differed significantly from the run time of the controls at two consecutive test points (Table 1). Increasing the dose to 60 mg/kg resulted in a prolonged duration of the effect throughout the whole experiment with a start 1 hr after the drug administration. Statistical differences as compared with the controls were reached at all but two test points (Table 1). One hr after the administration of protriptyline, 30–60 mg/kg, the decrease in run time seemed to be dose dependent.

A comparison in run time between the mice treated with chlorimipramine and mice treated with protriptyline showed statistical differences between the two groups only in the dose range of 7.5–15 mg/kg (Table 1).

DISCUSSION

Because of the great number of statistical testings (n=195) made in the present study some of the significant differences at the 5 and 2% levels probably can be explained by mass significance. That this is the case for the single significant difference obtained in the comparison between protriptyline and controls at the dose levels of 7.5 and 15 mg/kg (Table 1) seems almost certain. With regard to mass significance a convincing effect of protriptyline was seen only after a dose of 60 mg/kg although a dose of 30 mg/kg might have a small effect.

The general conclusion that chlorimipramine in the dose range of 7.5–60 mg/kg decreases the run time appears not to be invalidated by mass significance.

Chlorimipramine and protriptyline are both organic bases and are both highly lipophilic. Accordingly, maximal brain values are reported 0.25–0.5 hr after IV administration of chlorimipramine 5 mg/kg to rats [7]. The brain concentration then rapidly decreased. The 6 hr concentration was thus about 10% of the 0.5 hr concentration [6]. After IV administration of protriptyline, 1 mg/kg, to rats the maximal brain concentration was seen after 0.5 hr. (Data on file, Merck Sharp and Dohme, Research Laboratories, West Point, Pennsylvania). The concentration 6 hr after the protriptyline administration was in this case also about 10% of the 0.5 hr concentration. In mice protriptyline administered IP gave near maximal brain concentrations 0.5 hr after injection of the drug. (Data on file, Merck Sharp and Dohme, Research Laboratories, West Point, Pennsylvania). The time of the appearance and the duration of the decrease in run time obtained after the administration of chlorimipramine, 7.5–60 mg/kg, and protriptyline, 30–60 mg/kg, seem thus to be re-

lated in time with the reported high brain concentrations of the two drugs.

Chlorimipramine is a relatively specific blocker of the amine pump in 5-HT neurons. The ED_{50} for this blockade is 7 mg/kg while the ED_{50} for the blockade in NA neurons is over 25 mg/kg [2, 3, 4]. The converse is true for protriptyline, the ED_{50} for the blockade in the NA neurons and the 5-HT neurons being 4 mg/kg and over 25 mg/kg respectively. The minimal effective dose of chlorimipramine to decrease the run time was 7.5 mg/kg. This dose is very similar to the ED_{50} for the blockade of the amine pump in the 5-HT neurons. Protriptyline, however, at a dose of 15 mg/kg, which is several times higher than the ED_{50} for the blockade of the amine pump in the NA neurons, did not influence the run time. These findings suggest that 5-HT has an important role in tube running activity. A dose of protriptyline, which causes maximal blockade in the NA neurons, about 6.25–12.5 mg/kg IP, [3] did not decrease the run time. The observed effect after protriptyline, 60 mg/kg, can be explained by a blockade of the pump in the 5-HT neurons. The dose of this compound giving a 50% blockade of the amine pump in the 5-HT neurons can at least be supposed to be higher than 25 mg/kg IP [2].

After increasing the chlorimipramine dose no further decrease in the run time during the first 2 hr after the administration of the drug was observed. This finding is in good agreement with the observation that various doses of chlorimipramine 7.5–60 mg/kg IP decreased the synthesis of 5-HT to the same extent [11]. The decreased synthesis is probably secondary to the blockade of the uptake mechanism at the cell membrane of the central 5-HT neurons. This blockade causes increased receptor activation postsynaptically [8]. Probably via a feed-back mechanism the impulse activity in the presynaptic 5-HT neurons is decreased [1,15] and the synthesis and/or turnover of 5-HT reduced [5, 9, 16].

Apart from initial screening test [17] very few data have been reported on the behavioural effects of chlorimipramine administered alone. Chlorimipramine but not protriptyline has been shown to potentiate the increased motor activity recorded in mice treated with a peripheral decarboxylase inhibitor and 5-hydroxytryptophan [10]. This increased motor activity was mainly ascribed to activation of central 5-HT receptors.

We have earlier demonstrated a dose dependent (6.25–25 mg/kg) decrease in run time after 5-HTP administration to nialamide pretreated mice and a dose dependent (50–200 mg/kg) decrease after treatment with only nialamide [14]. In addition LSD was found to give a short-lasting but marked decrease in run time. By increasing the dose of LSD (2.5–10 mg/kg IP) this effect was not increased but prolonged [13]. These results together with the findings of the present investigation seem to be indirect evidence that 5-HT has a role in tube runnning activity.

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