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BRIEF COMMUNICATION

Effect of Daily Saline, Drug or Blank Injections on the Susceptibility to the Convulsant Effect of Drugs

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IZQUIERDO, I., J. FERNANDES, R. OLIVEIRA AND F. SETTINERI. *Effect of daily saline, drug or blank injections on the susceptibility to the convulsant effect of drugs.* PHARMAC. BIOCHEM. BEHAV. 3(4) 721–722, 1975. – The daily intraperitoneal injection to rats of doses of metrazol (30 mg/Kg), strychnine sulfate (1 mg/Kg) or picrotoxin (1.2 mg/Kg) that were initially subconvulsant, caused after a number of days which varied with the drug, clonic convulsions in a high percentage of the animals. However, after 18 daily injections of saline there was a similar increase of seizure susceptibility to the 3 drugs. The daily handling of rats as for injection, either followed or not by actual abdominal pricking (blank injection), had a similar though less pronounced effect. In animals that were housed in the same room where the others were tested, but which were not handled, the above mentioned doses of metrazol, strychnine and picrotoxin had no convulsant effect. These results indicate that the procedure of submitting rats to daily intraperitoneal injections is not as unconsequential as is usually thought to be, and that it may induce neurological changes.

Daily injection Seizures Metrazol Strychnine Picrotoxin Kindling

THE procedure of submitting rats to daily intraperitoneal injections is very widely used. It involves a series of manipulations (grasping the rat, turning it over, pricking, introducing a jet of a relatively cool liquid into the abdomen) that are not necessarily harmless and which may eventually constitute a sequence of aversive stimuli [1,4]. Thus, even daily saline injections may cause changes of both contingent (i.e., maze runs preceding injections, [1]) and non-contingent (i.e., maze runs made several days after the injections, [6,7]) behavior, as well as certain neurochemical alterations [3,4].

METHOD AND RESULTS

The present observations started as a study of possible kindling with drugs in rats. Kindling consists of the lowering of the seizure threshold of limbic or other brain areas as a result of daily or otherwise repeated subthreshold stimulation [2,5]. We set out to see if a similar phenomenon could be obtained with daily intraperitoneal injections of metrazol (30 mg/Kg), strychnine sulfate (1 mg/Kg) or picrotoxin (1.2 mg/Kg) to adult female rats (135 to 176 g). These doses of the drugs are widely known to be subconvulsant. Control animals received 1 ml/Kg of saline, this being the injection volume in all cases. Each group had 20 rats and injections were at 2 to 4 p.m. After a number of days which varied with the drug, clonic (rarely tonic) convulsions occurred within 15 min of the injection of all of the 3 drugs (Fig. 1). Since during the first several days of treatment there were no convulsions, this was quite suggestive of kindling. The number of days up to the first of 2 consecutive days with convulsions was of 8.0 ± 0.4 for strychnine, 11.0 ± 1.2 for picrotoxin, and 15.6 ± 0.6 for metrazol (excluding those rats in each group which never convulsed, see Fig. 1). All differences between groups were significant in a Duncan multiple range test at a 0.005 level. This suggests that, at the doses tested, strychnine had a more powerful kindling effect than picrotoxin, and this in turn than metrazol.

However, the rats that were injected during 18 days with saline also displayed clonic convulsions when treated with any of the 3 drugs, at the doses mentioned above, on the 19th day (7 received strychnine, 6 picrotoxin and 6 metrazol) (Fig. 2). This suggested that at least part of the lowering of seizure threshold was due to the injection procedure itself, and not to the drugs. Three additional groups

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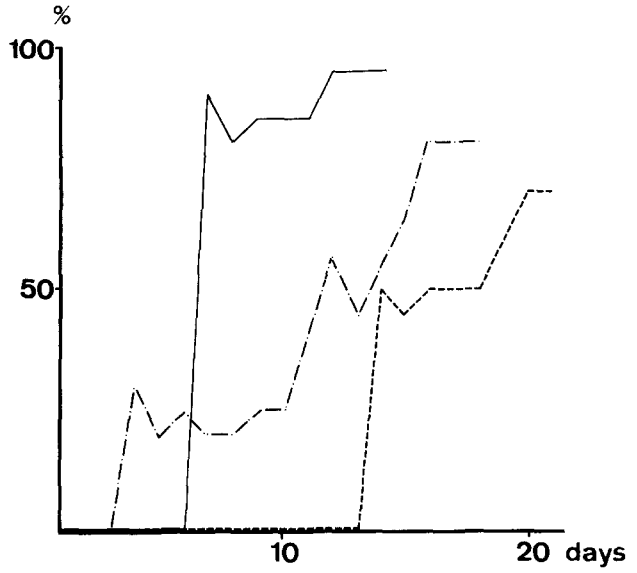

FIG. 1. Percentage of rats developing clonic convulsions (ordinates) to initially subconvulsant doses of strychnine sulfate (1 mg/Kg, ——), picrotoxin (1.2 mg/Kg, –·–), or metrazol (30 mg/Kg, – – –) given daily intraperitoneally (number of days in abscissa).

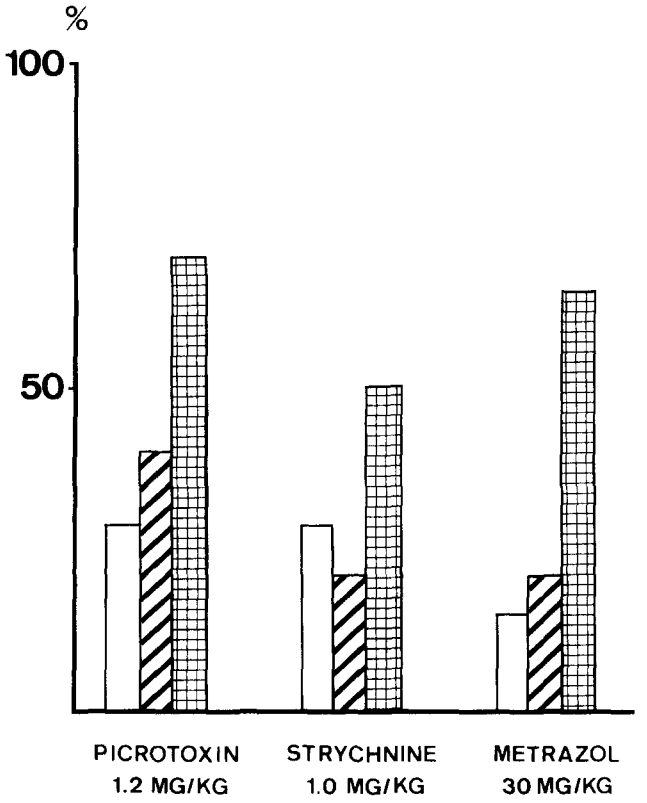

FIG. 2. Percentage of rats showing clonic convulsions upon intraperitoneal injections of strychnine, picrotoxin and metrazol at the doses indicated in Fig. 1. Open blocks: rats handled daily during 18 days as for intraperitoneal injection but not pricked. Dashed blocks: rats receiving 18 daily blank injections. Squared blocks: rats which received 18 daily consecutive saline (0.1 ml/Kg) injections. The spectator group (see text) is not illustrated here since it displayed no convulsions.

of 20 rats were used: one was handled every day as for injection during 18 days (picked up, grasped, turned over, given a tap with the finger on the abdomen); another was similarly handled and then pricked in the abdomen with a 27 ga needle (the same used for injections) (blank injection group); the third group was not handled but was housed in the same room where other rats were being convulsed every day, so that they could smell, hear or even see them. All groups received on the 19th day an injection of strychnine (7 rats per group), picrotoxin (6 rats per group) or metrazol (6 rats per group) at the doses stated above. A small percentage of the animals in the handled and in the blank injection group showed convulsions; none of the unhandled "spectator" rats did (Fig. 2). This suggested that the manipulations involved in the injection procedure were instrumental in bringing about the lowering of seizure threshold to the drugs.

DISCUSSION

Thus, the initial objective of detecting kindling with convulsant drugs was attained, but at least a part of the kindling effect seemed to depend on the injection procedure rather than on the drugs themselves. Since, however, these differed in their effectiveness to induce kindling, possibly some of the kindling which they caused may be considered to be due to intrinsic properties of the drugs.

The present data show that the daily handling of rats as for injection, or the daily blank or saline injection may cause, in addition to the previously reported changes in contingent [1] and non-contingent [6,7] learned behavior, a lowering of their seizure threshold. It is not known if any of these effects relates to each other, or to the neurochemical effects that daily saline injections are known to produce (an increase of hippocampal RNA concentration, [3,4] and a decrease of thalamic ATP concentration [3]).

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