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A simple, non-invasive method for the measurement of reserpine--induced tremor in rats

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Technical communication

Abstract. A new, non-invasive method for measuring reserpine-induced tremor in rodents is described here. The test procedure is based on the piezo-electric principle and was evaluated using the tremorogenic compound reserpine and the stereotypies-inducing drug apomorphine. Whereas for reserpine an orderly and dose-related increase in activity was observed, no such effect was detected with apomorphine. In order to further evaluate the test procedure, studies on the antagonism of reserpine-induced tremor were also performed. Results from these studies indicated that the DA-agonist lisuride, but not the S_2-antagonist ritanserin, were able to antagonize the reserpine-induced tremor in a dose-related manner.

Key words: reserpine, tremor, lisuride, automated test procedure

INTRODUCTION

Methods for evaluating drug-induced tremor in rodents are mostly based on visual observation. This implies that the presence or absence of tremor is scored along a rating scale (e.g. Goldstein et al. 1975, Dickinson et al. 1983). Disadvantages of such observation methods include: (1) the insensitivity to relatively small changes in tremor activity, (2) the possible bias of the observer, and (3) the sometimes relatively large group of animals that is needed to obtain reproducible results. Attempts to assess tremor more objectively were unsatisfactory till now because either the animals had to be restrained (e.g. Gothoni et al. 1981) or some equipment had to be attached to the animal (Hallberg et al. 1985, Shinozaki and Hirate 1986).

We report here on a method allowing to record tremor in freely moving rats using the piezo-electric principle (Marcus 1982, Megens et al. 1987). The method is based on a quantitative analysis of the voltage outputs derived from deformations of two pieces of piezo-film attached to the floor of an animal test cage. The utility of the test procedure was evaluated using the tremorogenic compound reserpine (Glow 1959, Morrison and Webster 1973). Furthermore, the possible antagonism of the reserpine-induced tremor with the $5HT_2$-antagonist ritanserin (Janssen 1985) and the DA-agonist lisuride (Clark et al. 1985), were studied. To exclude the idea that different forms of stereotyped behaviour also would induce a high activity in this test procedure, different doses of apomorphine were also tested.

METHODS

Animals

Female Wistar rats weighing 200-220 g were used. The rats were food deprived for 24 h and housed in groups of 12. After the first treatment (see below), the rats were housed singly in standard rodent observation cages. All experiments were carried out between 1 and 5 p.m. in an air-conditioned laboratory (temperature 21 $\pm$ 1°C; R.H. 65 $\pm$ 5 %). The animals were used only once.

Test apparatus

TEST CAGE

The test cage consisted of a Plexiglas chamber measuring 25 x 24 x 25 cm with a covering roof of Plexiglas fitted with holes to allow ventilation. The floor of the test cage consisted of a flexible Plexiglas plate (45 x 23 x 0.6 cm) which was centered underneath the cage. The test cage did not contact onto this floor plate. The floor plate rested at its four corners on a rubber point of support. Two pieces of piezo-film (200 x 100 x 0.025 mm, polymeric PVF2) were tied up parallel next to each other underneath the middle of the floor plate, and covered with a sticking protection foil. Connections were made from both piezo-films to an amplifier, using a clip connection attached on each piezo-film.

The test cage was situated in a sound and light attenuating outer box (Coulburn Instruments®), being constantly illuminated and air-ventilated.

ELECTRONIC DEVICE

A schematic draft of the electronic device is given in Fig. 1. A short technical description is given here. The piezo-electric response, produced by the deformation of the cage floor is amplified by an individual amplifier (A1, A2) for each piezo-film separately. The sum of these signals is processed by a noise detection system which prevents further transmission if the signal is below the selected noise level of 100 mVolt. Practical evaluations determined a band-pass filter between 6 and 12 Hz, with an 18 db/oct. slope. After filtering the trigger unit detects the occurrence of a response and in the meantime, the positive peak detector clamps the amplitude of the response. Transferred to analog memory 1, this amplitude can be compared with the amplitude of the previous response saved in analog memory 2. If the absolute value of the difference of these pulses is lower than the selected pulse difference of 400 mVolt and the pulse arrives within a time window less than 167 ms (6 to 12 Hz filter), the interpulse detector generates a clock pulse for the pulse/response counter (called programmed counter). Otherwise, the programmed counter is reset. Depending on the valid of the presetable prescaling factor N for this programmed counter, the reserpine tremor counter will increase with one count after N valuable successive responses. Once done, the programmed counter is reset. In the here described experiments, the prescaling factor was set at 10. This thus implies that the reserpine tremor counter was only triggered once if 10 successive pulses occurred, differing no more than 400 mVolt from each other, as compared in pairs of two pulses. The reserpine tremor counter value is continuously on display and can be sent to a computer for further data analysis.

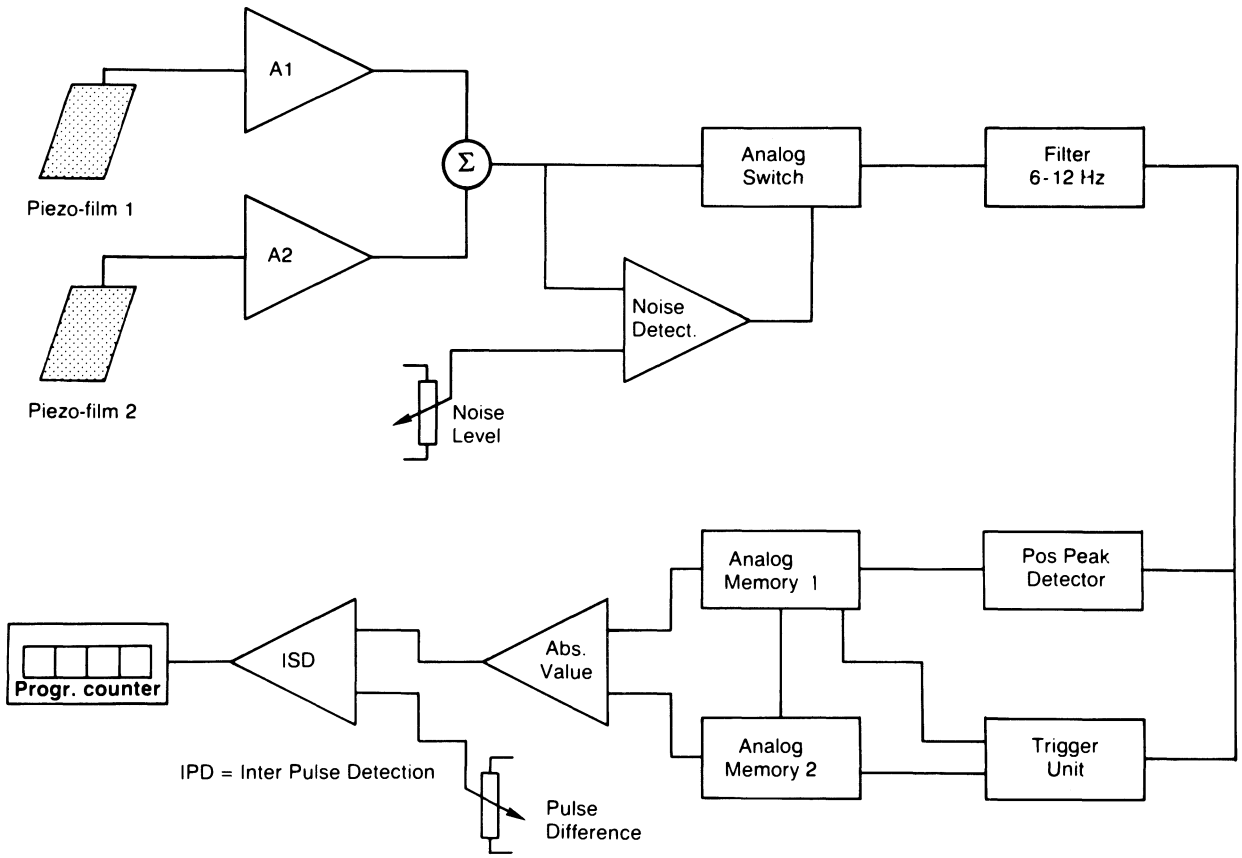

Fig. 1. Schematic design of the electronic device used in the reserpine tremor test. For further information the reader is referred to the section Methods.

TEST PROCEDURE

Generally, at a specified time after treatment (see below), rats were individually placed in the test cage and tremor activity was measured continuously during a 15-min test session. After the test session, the animal was removed and the test box cleaned.

In order to determine a dose-response function for reserpine, rats were given an intravenous (i.v.) injection of either 0.5, 1.0 or 2.0 mg/kg reserpine and tested 60 min later. To characterize a time-response relationship, rats were treated intravenously with 2.0 mg/kg reserpine and repeatedly tested at 0, 60 and 120 min after treatment. In both experiments, control animals received a vehicle injection.

In the experiments on the antagonism of reserpine-induced tremor, rats were pretreated intragastrically with a test compound at 90 min before testing. This pretreatment was followed by an i.v. injection of 2.0 mg/kg reserpine at 60 min before testing. Control animals received a combined treatment of either saline plus reserpine (i.e. SR group) or of saline plus the vehicle of reserpine (i.e. SV group).

In a last experiment, rats were injected subcutaneously with different doses of apomorphine and tested 60 min later.

At all experimental conditions the test results of 5 rats were collected except for the SR and SV group in the double treatment conditions, for which the results of 20 rats were collected.

DRUGS

The compounds used were: reserpine (Janssen Chimica, Belgium), ritanserin, apomorphine HCl (Sandoz, Switzerland) and lisuride maleate (Schering AG, West Germany). All compounds were dissolved in water except for reserpine for which ascorbic acid was added in an amount (w/w) which was four times that of reserpine.

All intragastric and subcutaneous treatments were administered in a volume of 1.0 ml/100 g b.w.. For the i.v. injections, a volume of 0.2 ml/100 g was used. The

doses tested for each compound are specified in the Results' section.

STATISTICS

The Mann-Whitney U-test, two-tailed (Siegel 1956), was used to evaluate possible differences between experimental conditions.

RESULTS

The 5 rats that received an i.v. injection of the vehicle of reserpine at 60 min prior to testing revealed a mean (± SEM) activity score of 43.6 (± 5.1) counts. Reserpine, tested at 0.5, 1.0 and 2.0 mg/kg, dose-related increased ($P<0.05$) the average activity rate to a maximal effect of 243.0 (± 22.3) counts at 2.0 mg/kg (Fig. 2, left panel). Because 2.0 mg/kg reserpine robustly increased activity above the vehicle control level, this dose was further used to determine a time-relationship effect of reserpine.

Vehicle-treated rats average 19.5 (± 4.0) counts when placed into the test box immediately after the i.v. treatment (Fig. 2, right panel). The activity increased significantly ($P<0.01$) to 43.7 (± 5.1) counts during the second exposure at 60 min after treatment. At the third test, i.e. at 120 min after injection, the average activity was 45.3 (± 6.4) counts and no differences were observed ($P>0.05$) with the results obtained at the 60-min interval. The average activity scores of the rats pretreated with 2.0 mg/kg reserpine and tested at 0, 60 and 120 min after injection were 51.3 (± 11.7), 157.7 (± 36.0) and 156.3 (± 39.0) counts, respectively. Also for the reserpine-treated group, an increased activity ($P<0.01$) was apparent between the first and second exposure, while no differences ($P>0.05$) were observed between the results obtained at the 60- and 120-min test periods. At all three periods of time, the results of the 2.0 mg/kg reserpine group exceeded those of the vehicle controls ($P<0.05$). The difference between both groups was most pronounced at the 60-min interval.

Because rats treated with 2.0 mg/kg reserpine were tested at 60 min after treatment in both the dose-response and the time-relationship experiments, both data sets were compared with each other in order to have an idea of reproducibility. The mean activity score obtained with the two groups of 5 rats in both experiments ranged from 243.0 (± 22.3) to 197.7 (± 36.0) counts. No significant differences ($P>0.05$) between both groups were observed.

To exclude the possibility that any kind of stereotyped behaviour would result in a similar increased activity in this test procedure, a dose-response function for apomorphine was established in comparison with saline controls (Fig. 3). Sixty min after a subcutaneous injection of saline, the controls averaged 36.8 (± 2.9) counts. For 0.16, 0.63 and 2.5 mg/kg apomorphine, the corresponding mean values were 44.6 (± 3.3), 36.8 (± 5.4) and 13.8 (± 10.9) counts, respectively. No significant differences with the saline controls were found ($P>0.05$).

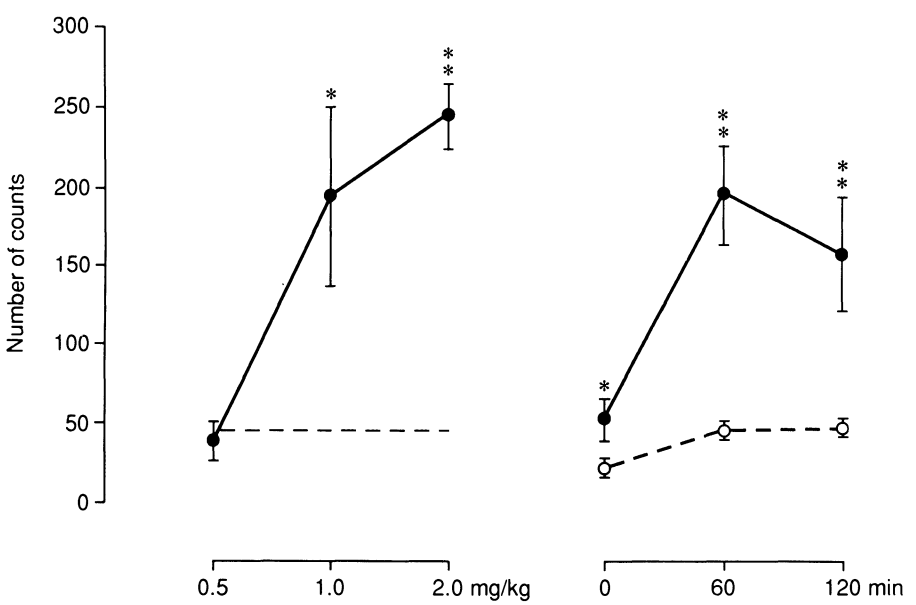

Fig. 2. Dose-response (left panel) and time-relationship (right panel) effects of reserpine in the automated reserpine tremor test. Each point represents the mean (± SEM) activity score of 5 rats. All injections were given intravenously at 60 (left panel) or at 0, 60 and 120 min before testing (right panel). The horizontal line (---) and the open circles (o---o) represent the corresponding vehicle controls. Differences between experimental conditions were evaluated using the Mann-Whitney U-test, two-tailed (Siegel 1956, $*P<0.05$; $**P<0.01$).

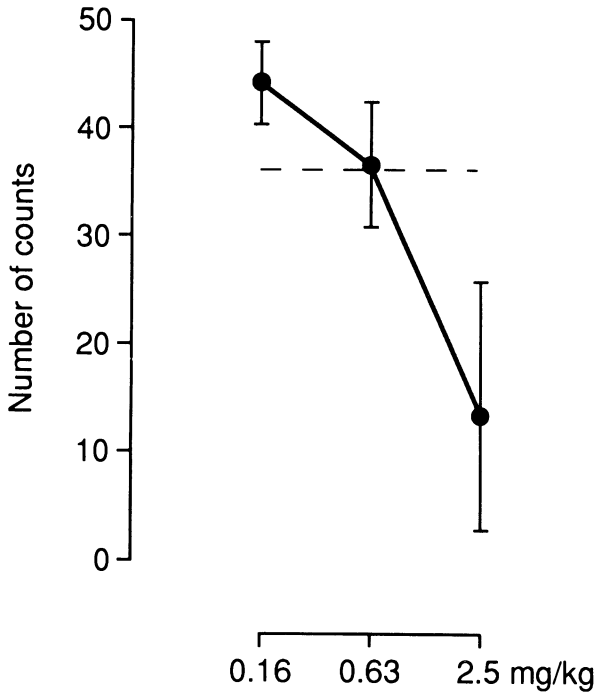

Fig. 3. Dose-response effect of apomorphine on tremor activity. All injections were given subcutaneously at 60 min prior to testing. Each point represents the mean (± SEM) results of 5 rats. The horizontal line indicates the saline control level. At no time were the differences between apomorphine and saline statistically significant (Mann-Whitney U-test, Siegel 1956, $P>0.05$).

In a last group of experiments, the antagonism of reserpine-induced tremor was studied. To do so, rats were treated at 90 and at 60 min prior to testing. Two control groups of 20 rats each, an SV and an SR group, were included in the study. The average activity score of the SV group was 34.3 (± 3.1) counts, whereas for the SR group it was 152.0 (± 15.1) counts. Thus 2.0 mg/kg reserpine clearly increased responding ($P<0.001$) above the saline control level. The two compounds tested, reduced the reserpine-induced tremor to some extent (Fig. 4). For ritanserin, limited effects on reserpine-induced tremor were observed at 40.0 mg/kg. Although at 40.0 mg/kg ritanserin a mean of 106.2 (± 30.3) counts was registered, no significant difference ($P>0.05$) with the SR group was obtained.

Lisuride, tested at doses ranging from 0.005 to 0.16 mg/kg, decreased the reserpine-induced activity in a dose-related manner. The first active dose of lisuride was 0.01 mg/kg. At this dose, an average of 90.6 (± 15.2) counts was measured. The average activity score further decreased as the dose of lisuride increased and at 0.04 mg/kg of lisuride there were still only 44.4 (± 10.1) counts. 0.04 mg/kg was also the first dose at which no difference ($P>0.05$) against the SV controls was apparent anymore. Increasing the dose of lisuride to 0.08 and 0.16 mg/kg resulted in further minor decreases of the activity score to 27.8 (± 5.0) and 31.4 (± 4.8) counts, re-

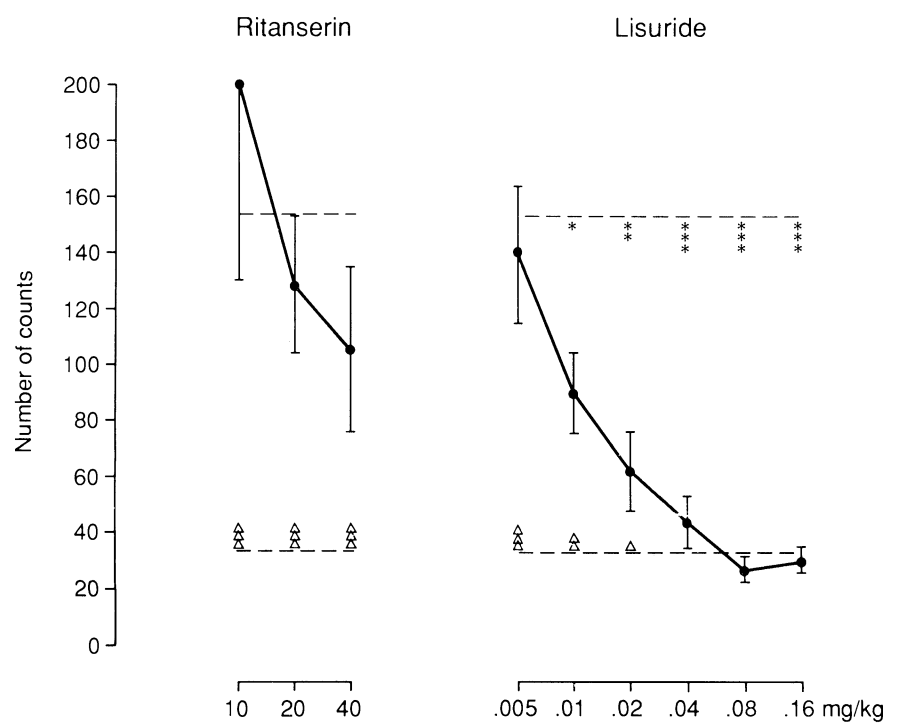

Fig. 4. Antagonism of reserpine-induced tremor with ritanserin and lisuride. Each point represents the average (± SEM) results of 5 rats. The horizontal lines indicate the saline-reserpine (upper line) and the saline-vehicle (lower line) control level, respectively. Both antagonists were infused intragastrically at 90 min prior to testing. 2.0 mg/kg reserpine or its vehicle was given intravenously at 60 min before testing. Differences from the saline-reserpine control level (*) and the saline--vehicle control level (D), were evaluated using the Mann-Whitney U-test, two-tailed (Siegel 1956, D, *: $P < 0.05$; DD, **: $P<0.01$; DDD, ***: $P<0.001$).

spectively, but basically a plateau level also observed in control recordings was reached.

DISCUSSION

In this article, a non-invasive method for the measurement of tremor in freely moving rats was described, based on the piezo-electric principle (Marcus 1982). Practically, two pieces of piezo-film are tied up underneath the floor of a test cage which was supported at the four corners so that it could bend freely. By adapting this technique, any activity exerted by a rat could be transferred into voltage outputs. These electrical signals can easily be processed according to a previously determined criterion. In this study, one tremor count represents the appearance of 10 successive electrical signals that, after having been amplified and filtered, all exceed a trigger level of 100 mVolt and differ no more than 400 mVolt from each other. A train of analog pulses was selected here to characterize tremor because visual observations of reserpine-injected rats indicated that tremor mostly appeared in bursts which, as a consequence, will bend the floor of the test cage continuously in a constant rhythm.

The utility of the test procedure was evaluated using the tremorogenic compound reserpine (Glow 1959) and the stereotypies-inducing drug apomorphine (Niemegeers and Leysen 1982). Both in terms of a dose-response and a time-effect relationship, an orderly function was obtained with reserpine. The differences between reserpine and vehicle-treated rats increased both in function of the dose of reserpine and the time interval between the injection of the drug and the test. For apomorphine, on the contrary, no dose-related increase in activity above the saline control level was obtained in this test procedure although stereotyped behaviours were visually observed, especially at the highest dose tested. These results thus indicate that a simple introduction of stereotyped behaviours is insufficient to augment responding in this automated test procedure. Hence, selectivity with regard to the measurement of reserpine-induced tremor seems to be achieved.

In a last group of experiments, it was tested whether the test procedure could be used to study the antagonism of reserpine-induced tremor in rats. A dose of 2.0 mg/kg reserpine was selected as the reference dose because at this dose tremor activity increased four to five fold from an average base-line of 34 counts. Ritanserin was without any significant effect on reserpine-induced tremor. Even at doses up to 40.0 mg/kg, no substantial activity decrease below the reserpine control level was apparent. Lisuride, on the contrary, antagonized the effects of reserpine in a dose-related manner to a level which was within the limits of the SV controls. The first active dose was 0.01 mg/kg and at 0.04 mg/kg, no differences from the SV controls were apparent anymore, indicating complete tremor block. Given these results on the antagonism of the reserpine-induced tremor, it can be stated that DA-agonists like lisuride are able to overcome the reserpine-induced tremor in rats. These results thus confirm earlier reports indicating that DA-agonists may overcome some of the symptoms induced by reserpine in rodents (Goldstein et al. 1975, Wagner and Anderson 1982, Anderson 1985). For ritanserin, no substantial antagonism of the reserpine-induced tremor was observed. Because ritanserin, a selective $5HT_{2A}$-antagonist (Janssen 1985), was unable to antagonize the reserpine-induced tremor, it might be concluded that $5HT_2$-antagonism is not sufficient to overcome the reserpine-induced tremor in rats.

Globally, the results presented here indicate that reserpine-induced tremor can objectively and reliably be measured in freely moving rats using the piezo-electric principle. The measurement appears selective in that tremor but not any type of stereotyped behaviour will be counted. This methodology might also be used to study other forms of induced tremor.

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