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Automated Method for the Measurement of Fentanyl-Induced Muscular Rigidity

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DELVECCHIO, R. A. *Automated method for the measurement of fentanyl-induced muscular rigidity.* PHARMACOL BIOCHEM BEHAV 46(2) 265-268, 1993.—An automated method is described that can accurately and reliably measure weight displacement resulting from hindlimb extension due to muscular rigidity following opioid administration in rats. IV administration of fentanyl (0.035 mg/kg) immediately induced rigidity. Rigidity was dose dependently reversed by the α_2-agonist clonidine with an ED_{50} value equal to 0.011 mg/kg IV. Second, rigidity was restored following administration of the α_2-antagonist idazoxan (0.3 mg/kg, IV) thereby confirming an α_2-mediated mechanism of action. Previously, reversal of fentanyl-induced muscular rigidity was measured by subjective rating criteria not suitable for quantitative potency comparisons. The new automated rigidity model provides a simple yet precise measurement of the ability of α_2-agonists to attenuate opioid-induced muscular rigidity in rats.

Fentanyl induced Muscular rigidity IV Clonidine Idazoxan

IT has been shown that α_2-agonist agents effectively reverse opioid-induced muscular rigidity (1,3). It was suggested that this reversal of rigidity was the result of a reduction of sympathetic outflow that was selectively effected by α_2-agonists (3). Originally, fentanyl-induced rigidity in rats was indicated by extreme extensor muscle tone and the absence of hindlimb flexion while the stiff animal was supported in a vertical position by the investigator. Rigidity was considered to be reversed if, within 2 min, the rat bent spontaneously at the abdomen when held vertically and the hindlimbs could be flexed passively by pushing on the rear paws while in a supine position. A quantal "yes-no" scoring system for rigidity was described (3) and later refined (1) according to a scoring system on a scale of 0-3 (0 = no rigidity, 1 = mild rigidity, 2 = moderate rigidity, and 3 = marked rigidity). All of these assessments involved qualitative judgements to be made by the investigator. However, these judgements proved suspect at times, especially when assessing transient reversals caused by sedative agents such as diazepam (8). The present investigation was undertaken to accurately quantify the assessment of opioid-induced muscular rigidity by employing a novel automated test apparatus. The effects of fentanyl alone, the α_2-agonist clonidine, and the α_2-antagonist idazoxan were assessed in this automated rat rigidity model.

METHOD

Male Sprague-Dawley rats (Hilltop Farms, PA), weighing 250-320 g, were group housed (five/cage) under standard laboratory conditions as outlined in the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1985) with a 12 L : 12 D cycle. Food and water were available ad lib.

Following a 30-min acclimation to the laboratory, rats were administered compounds IV into the lateral tail vein by means of an infusion catheter (Terumo Surflo winged infusion 25G × 3/4, 0.41-ml tubing volume). Fentanyl citrate (Sublimaze; 0.05 mg/ml, Janssen Pharmaceutica, Beerse, Belgium) was administered at 0.035 mg/kg, the ED_{100} dose previously determined (1) to effect loss of righting reflex (LORR) and muscular rigidity in rats. Clonidine (Sigma Chemical Co., St. Louis, MO) and idazoxan (Research Biomedical, Inc.) were dissolved in distilled water and administered in an injection volume of 1 ml/kg. Doses are expressed as percent base.

The anesthetized (rigid) animal was placed in a supine position on the test apparatus (see Fig. 1). The rat's hindpaws were fitted into stirrups (6-0 surgical silk, Ethicon, Inc., Somerville, NJ). The stirrups were attached to a tether line extending up over a freely moving cylinder situated on top of a lab jack (Fisher Instruments). The tether line descended to a 50-g weight positioned on the weighing bed of a Mettler PE 24 balance. The balance was equipped with printing capabilities (Mettler GA-44). The weight was modified with a small amount of copper wire, forming an eye through which the tether line was fed. The tether line then extended up again to a second lab jack at the opposite side of the scale. A horizontal force applied to the stirrups (caused by the distal extension of the hindpaws during rigidity) was translated into a vertical force at the weight. This caused a displacement of the weight from the balance that was measurable.

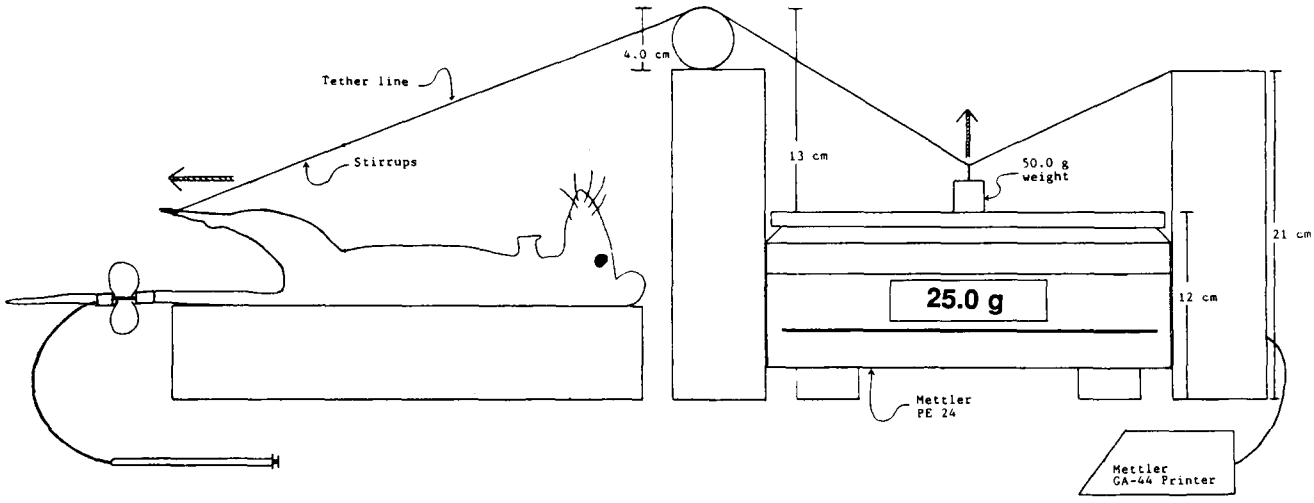

FIG. 1. Diagrammatic representation of a rigid rat following administration of fentanyl (0.035 mg/kg). This apparatus measures the displacement of weight caused by the distal extension of the rat's hindlimbs.

After the supine animal was secured to the stirrups, a timer was started. Doses of clonidine were administered in separate groups of rats (n = 5) 60 s after fentanyl administration. A positive control group was administered fentanyl alone (n = 7). At the highest dose of clonidine tested (0.08 mg/kg, IV), idazoxan at 0.3 mg/kg IV was administered 120 s after clonidine administration.

Data Analysis

Data was collected every 10 s after initiation of the test (i.e., fentanyl injection). Weight displacement for each fentanyl alone and fentanyl + clonidine dose group between 120 and 180 s was calculated as percentages of rigidity induced by fentanyl alone. This was done according to the following formula:

$$\% \text{ Reversal} = \frac{\text{fentanyl alone} - (\text{fentanyl} + \text{clonidine})}{\text{fentanyl alone}} \times 100$$

The ED_{50} value with 95% confidence limits for clonidine reversal of fentanyl-induced rigidity was calculated by means of the Litchfield-Wilcoxon method (5) using the six time points (10-s intervals) between 70 and 120 s after clonidine administration (130–180 s on the abscissa of Fig. 2).

RESULTS

Figure 2 shows a dose-dependent decrease in muscular rigidity (induced by fentanyl) by increasing concentrations of clonidine. The onset of clonidine reversal of fentanyl-induced rigidity was not immediate, making necessary the evaluation of an ED_{50} from 70 to 120 s after clonidine injection. During this time period, the ED_{50} is equal to 0.011 (0.008–0.015) mg/kg IV.

Figure 3 shows that the α-antagonist idazoxan at 0.3 mg/kg IV completely restored rigidity in the fentanyl + clonidine (0.08 mg/kg) group at 180–240 s.

DISCUSSION

This automated method, which is able to measure the effects of agents modulating fentanyl-induced muscular rigidity, is an improvement over previous subjective ratings or quantal observational methods (1,3). In this new model, muscular rigidity can be objectively and accurately measured by means of weight displacement resulting from fentanyl-induced hindpaw extension. Also, measurement of weight displacement enables the investigator to generate ED_{50} values for comparison of potency of various agents. Further, onset of drug action for reversal of opioid-induced muscular rigidity can be observed and recorded. In addition, mechanism of action of putative α_2-agonist compounds that reverse opioid-induced rigidity can be verified by challenge with an α_2-antagonist such as idazoxan, which reestablishes rigidity. A further advantage of this automated rigidity model is that it utilizes common laboratory equipment (balance, lab jacks, weight, string, dowel, etc.) that can be readily assembled and disassembled in the laboratory.

It was previously shown that the reversal of opioid-induced muscular rigidity was relatively selective for α_2-agonists (3) by generally decreasing sympathetic tone (2,4). The selectivity of these agents were differentiated from α_1-antagonists and β-antagonists that also reduce rigidity, but only at higher doses, producing CNS depression resulting in overall behavioral sedation and flaccidity (1). An automated rigidity paradigm that is both sensitive and selective for low doses of agents that do not produce CNS depression can be useful for determining the mechanism of action of α_2-agonists and other novel compounds that may also reduce muscular rigidity. Further, this automated model is an easy and simple method to establish in the laboratory that accurately and reliably measures muscular rigidity in rats.

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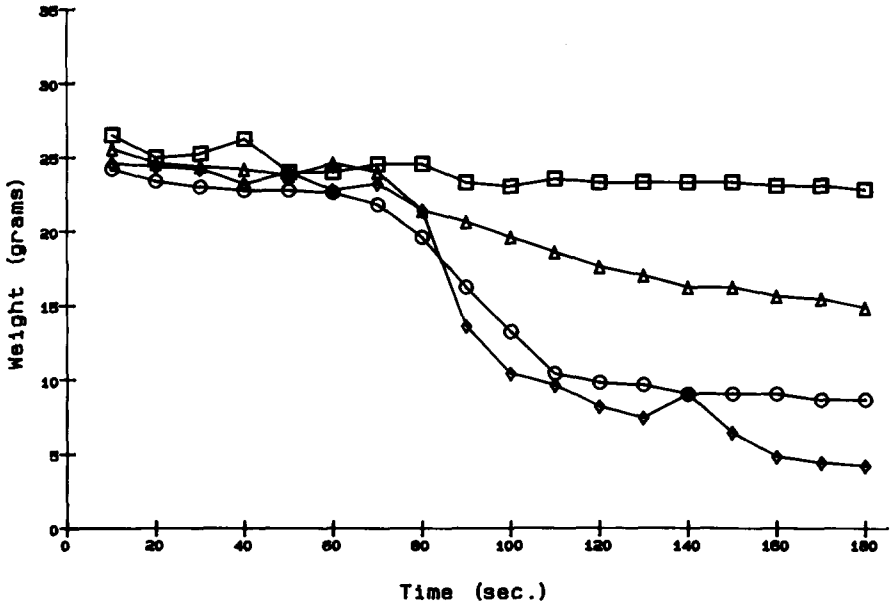

FIG. 2. Rats (n = 5–7) were initially administered fentanyl (0.035 mg/kg, IV) followed by IV administration of clonidine 60 s after fentanyl administration. The automated method for measurements of opioid-induced rigidity in rats was used to assess the intensity and modulation of rigidity over time. (□ – □), fentanyl alone (0.035 mg/kg); (△ – △), fentanyl and clonidine (0.005 mg/kg); (○ – ○), fentanyl and clonidine (0.02 mg/kg); (◇ – ◇), fentanyl and clonidine (0.08 mg/kg).

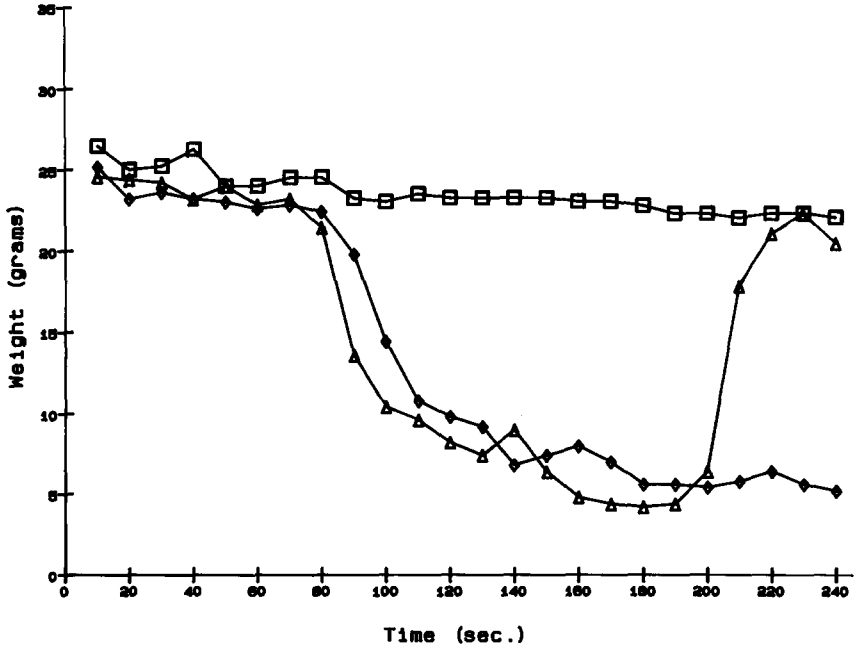

FIG. 3. Rats (n = 5–7) were initially administered fentanyl (0.035 mg/kg, IV) followed by clonidine (0.08 mg/kg) 60 s after fentanyl, then idazoxan (0.3 mg/kg) 120 s after clonidine. The automated method for measurements of opioid-induced rigidity in rats was used to assess the intensity and modulation of rigidity over time. (□ – □), fentanyl alone (0.035 mg/kg); (△ – △), fentanyl and clonidine (0.08 mg/kg) followed by idazoxan (0.3 mg/kg); (◇ – ◇), fentanyl and clonidine (0.08 mg/kg).

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