A Novel Approach for the Determination of the Pain-Producing Potential of Intravenously Injected Substances in the Conscious Rat

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The purpose of this study was to validate an experimental method for quantifying the pain producing potential of intravenously administered solutions. Response was measured in a Broome restraint tube modified by the addition of strain gauges. Struggling caused flexion of the tube, changing strain gauge output and increasing output variance. In experiment 1, five groups of 10 male Sprague Dawley rats were given intravenous injections of 1 ml of saline, acetate, HCl, citric acid vehicles, or KCl over a 1-min period. Results showed significant increases in output variance between saline and treated groups during the infusion period. In experiment 2, five groups of five rats were given intravenous injections of saline or 0.1, 0.05, 0.025, or 0.0125 *M* KCl. Rats responded in a dose-dependent manner, demonstrating the sensitivity of this technique. In experiment 3, two groups of four rats were given injections of morphine sulfate (2 or 4 mg/kg, ip) prior to administration of 0.05 *M* KCl. Two additional groups received no pretreatment prior to administration of saline or 0.05 *M* KCl. Results demonstrate that morphine ablates the response to intravenous administration of KCl. This model provides information concerning the pain producing potential of intravenously delivered compounds or formulations.

KEY WORDS: pain; intravenous; variance; rat; conscious.

INTRODUCTION

The measurement of pain in animal models has always posed problems to researchers. Generally, tests rely on behavioral reactions to stimuli that are presumed to be painful to animals. In addition, some tests rely on the experimenters' subjective assessment of an often variable series of responses. Some of the reactions to noxious stimuli that have been used to assess pain reactions include reflexive escape responses (tail-flick test, hot-plate test, pinch test), conscious escape (flinch-jump test), prolonged protective activity (fleeing or fighting), and retreat and withdrawal (1).

Recent toxicology studies undertaken in our laboratory showed that rats reacted to intravenous infusion of new compounds and/or vehicles by vocalizing and struggling in the restraint tube. Together, these signs of discomfort have been interpreted as an indication of pain caused by the test substance and/or vehicle. The purpose of this study was to develop a screening process to objectively evaluate the pain produced during iv administration of new compounds and formulations. A search of the current literature revealed no *in vitro* model which could be used to study this complex phenomenon. Results of these studies may be useful in (i) determining possible adverse effects of new compounds and/or vehicles early in the development process, (ii) predicting human response to infusion of intravenously administered materials, and (iii) development of materials and methods which do not produce pain upon intravenous administration.

MATERIALS AND METHODS

Rats were cared for and used in accordance with the *Guide for the Care and Use of Laboratory Animals,* DHEW Publication (NIH) 85-23, 1985, and subsequent amendments, and protocols were reviewed and approved by the Corporate Animal Welfare Committee.

Test substances were administered to a rat placed in a large Broome restraint tube (7.5-cm outside diameter) which had been modified to respond to the activity of the rat (see Fig. 1). The modifications were of two different types. First, a slot was cut halfway up the junction between the tube and the end partition. The remaining portion of the junction was reinforced with an epoxy filler. This modification allowed the lower portion of the tube greater flex in response to the rat's activity. Second, an electronic circuit, a strain gauge bridge, was constructed on the surface of the tube to monitor the tube's flex. Flexion of the tube changes the resistance through the strain gauge, resulting in a measurable change in voltage across the strain gauge bridge. Strain gauges (Type DDP-350-500 semiconductor, Kulite Semiconductor Products, Inc., Richfield, NJ) were attached approximately 2 cm from the base of the restraint tube. Power was supplied to the gauges and output was electronically amplified. Output was recorded directly on a personal computer via a data acquisition board (Model DT 2805, Data Translation, Inc., Marlboro, MA) and appropriate software (Labtech Acquire, Laboratory Technologies Corp., Wilmington, MA). The system was calibrated by suspending the restraint tube horizontally and attaching weights by means of a wedge inserted into the break in the tube at a specified distance from the end. Each weight was inserted 10 times and the response measured (see Fig. 2).

In order to distinguish pain induced by dosing solutions from pain due to needle stick, a polyethylene cannula (PE-10, Clay Adams, Parsippany, NJ) was inserted into the lateral tail vein as previously described (2). Rats were allowed to recover for a minimum of 2 hr after cannula placement prior to injection. Rats were placed in the restraint tube and allowed to become acclimated to the environment for 3 min. Immediately following the acclimation period, 1 ml of test solution was administered over 1 min. Response to injection was measured by calculating the variance (variation around the mean strain gauge output for each response interval) of the output (10 samples/sec) during the 1-min time period

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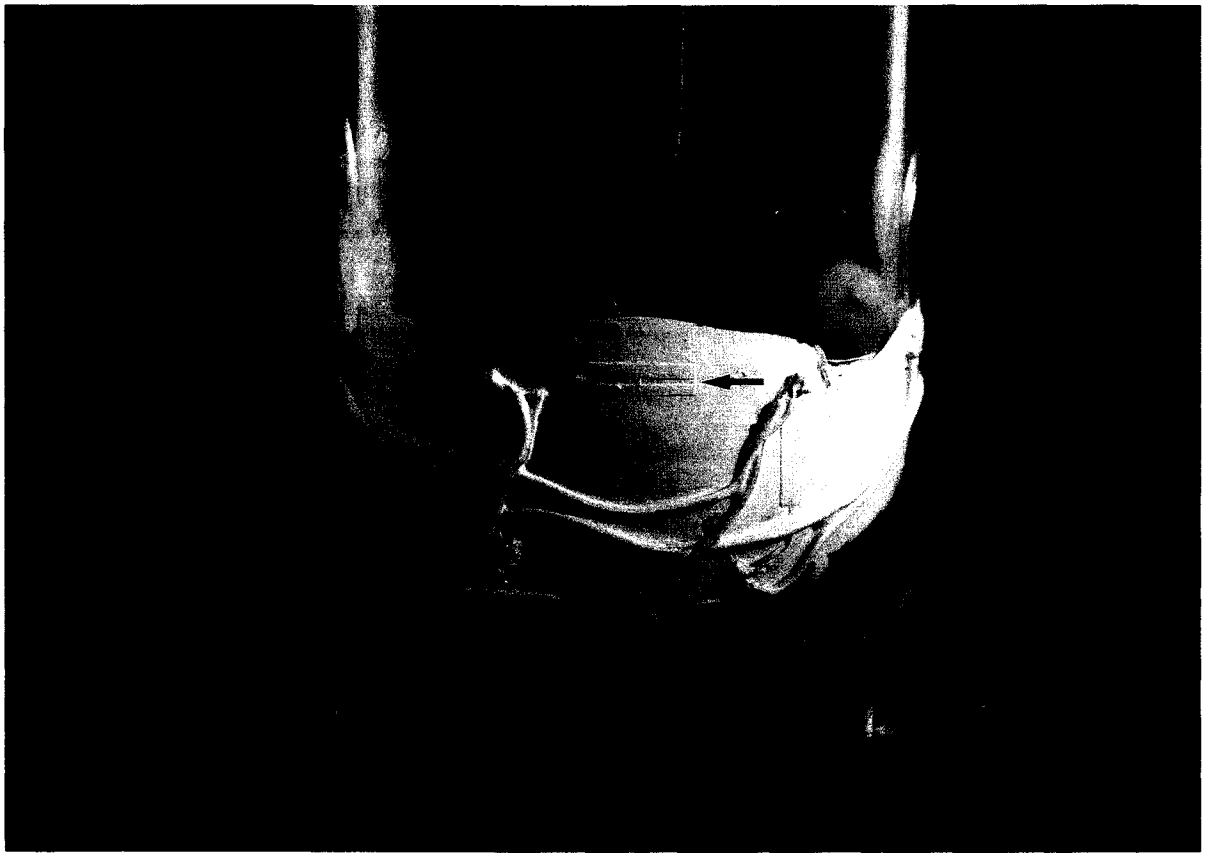

Fig. 1. Modified Broome restraint tube used to detect flexion of tube during administration of test solutions. Arrowhead denotes area which was cut to allow greater flex in response to the rat's activity (epoxy filler reinforcement shown; area which was cut is on opposite side). Arrow shows the strain gauge bridge on the tube's surface.

immediately preceding the injection and during the 1-min infusion time period. Because of the possible effects of learning on response, animals were dosed once and were not reused.

Experiment 1

Five groups of 10 male Sprague Dawley rats [Crl: CD(BR), Charles River Laboratories, Portage, MI; approximately 400 g each] were given a single intravenous injection of saline control or one of several solutions known to produce clinical signs of pain in rats and/or humans (anecdotal evidence). Substances tested included a sodium acetate vehicle (1 ml contained 2.42 mg sodium acetate, 0.00184 ml glacial acetic acid, 6.955 mg sodium chloride, q.s. ad water for injection USP, pH adjusted to 4.51 with 10% sodium hydroxide), an HCl vehicle (a 10% solution of HCl in water for injection USP, sufficient to make a 0.05 *N* solution, pH 1.3), a citric acid vehicle (1 ml contained 4.5 mg sodium chloride, 0.936 mg sodium citrate, 3.84 mg citric acid in water for injection USP, sufficient to make a 0.02 *M* solution, pH 2.8), and 0.1 *M* KCl (1 ml contained 7.45 mg KCl in water for irrigation USP, pH 4.7). Five animals were dosed each day, one from each of the five treatment groups. All reagents used were analytical or reagent grade.

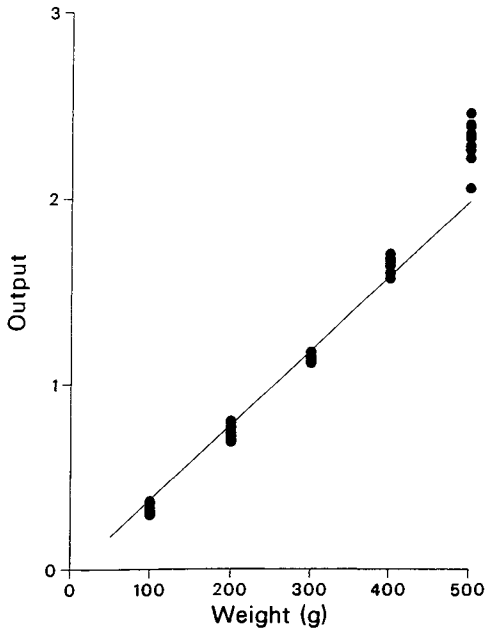

Fig. 2. Calibration of modified Broome restraint tube. Tube was suspended horizontally and weight suspended at a specified distance from the end.

Experiment 2

In order to investigate the sensitivity of the system, five groups of five male Sprague Dawley rats each received a single intravenous injection of saline control or 0.0125, 0.025, 0.05, or 0.1 *M* KCl. Rats were prepared and substances were administered as described above.

Experiment 3

The objective of this study was to evaluate the response of rats given an analgesic substance (morphine) prior to exposure to intravenous administration of a substance known to produce clinical signs of pain (KCl). Two groups of four male Sprague Dawley rats were given intraperitoneal injections of morphine sulfate (2 or 4 mg/kg) 15 min before administration of 0.05 *M* KCl. Two additional groups received no morphine pretreatment prior to administration of saline control or 0.05 *M* KCl. Rats were prepared and substances were administered as described above.

Statistical Analyses

Preliminary examination of the data from experiment 1 indicated departures from normality and heterogeneous variances among treatment groups. Therefore, log transformations were made on data from all experiments and treatment group differences were analyzed using analysis of variance. For statistically significant variables, treated groups were compared to the saline control group using the least significant difference method.

RESULTS AND DISCUSSION

Measurement of pain is a topic which causes problems for researchers not only from its scientific perspective, but from moral and ethical perspectives as well. It should be emphasized that during the design and conduct of this study, efforts were made to minimize pain experienced by the animals without compromising scientific endeavors. The length of time animals were exposed to potentially noxious substances, as well as the substances themselves, was chosen to provide valid data but not unduly stress the animal. The number of animals used was also minimized while still providing scientifically and statistically valid results. Adoption of this screening process in the development of new compounds or formulations may be useful in avoiding unnecessary pain in animals and humans.

Several parameters were considered as the response variable during initial examination of the data. Chief among these were the area under the curve (AUC), frequency of peaks, and variance of the data set. As data were being collected, it became apparent that AUC was not always an appropriate measure of the responsiveness of an individual. In several instances, changes in baseline output were observed during the infusion period because of the ability of an animal to shift its body position within the restraint tube. Lowered baseline values resulted in decreased AUC measurement which could lead to incorrect negative interpretation of the data when subjective analysis indicated a positive response. Similarly, frequency of peaks as a response variable was rejected due to inadequate definition of peaks. Animals which reacted positively to infusion of the test material struggled in the tube as a result of the discomfort and pain of the injection. Increased movement caused fluctuation in output and a concomitant increase in variance of the data set. Therefore, variance (variation of the data set around the mean strain gauge output for each response interval) was a more accurate indicator of the rat's reaction to administration of the test substance than the other variables. Typical negative (saline) and positive (acetate vehicle) individual responses are presented in Fig. 3.

Mean output variances for each dose group in experiment 1 are presented in Fig. 4. No difference was observed between treated and control groups during the pretreatment period ($P = 0.6216$); however, variance of treatment groups 2–5 was significantly greater than controls ($P = 0.0006$) during the infusion period. Increased variance was interpreted as an indication of greater movement in the tube due to increased discomfort and pain in response to the injection.

Results of experiment 2 are depicted graphically in Fig. 5. Statistical evaluation of results shows no significant difference in strain gauge output between treatment groups during the pretreatment period. All treated groups show a significantly increased response relative to controls during the injection period, and the intensity of the response increased with increasing dose. Within the KCl groups, only 0.10 vs 0.05 *M* and 0.025 vs 0.0125 *M* were not statistically different from each other. In this experiment, rats responded in a dose-dependent manner, demonstrating that the technique has an adequate sensitivity to discriminate between varying concentrations of an irritating material.

Results of experiment 3 are depicted graphically in Fig. 6. No significant difference in strain gauge output between

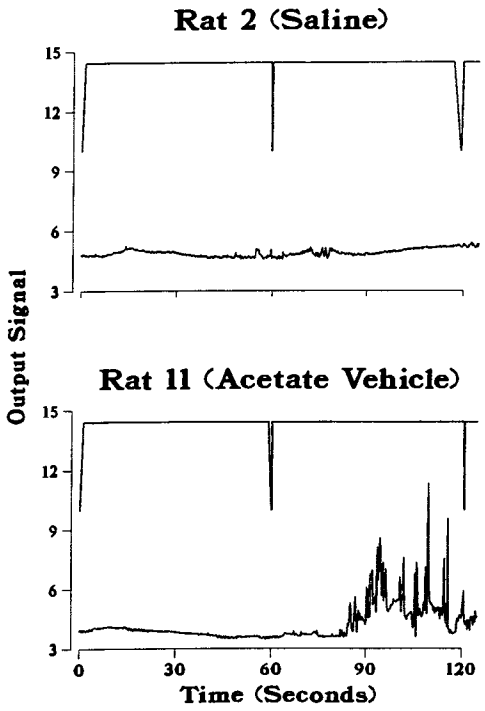

Fig. 3. Response of rats 2 and 11 to infusion of 1 ml of saline and acetate vehicle, respectively. The top line represents the event marker and indicates the beginning of the pretreatment period and the beginning and end of the infusion period.

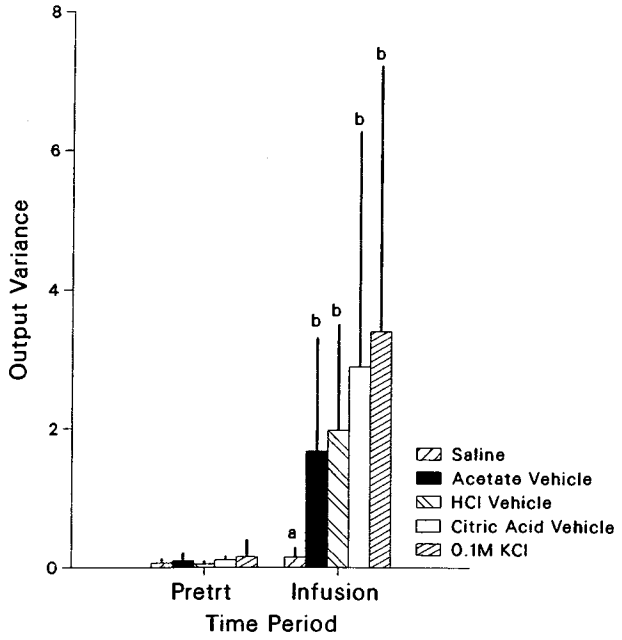

Fig. 4. Mean output variance of strain gauge output for experiment 1. Time period refers to pretreatment (Pretrt) and infusion periods (Infusion). Bars with different letters differ ($P < 0.01$).

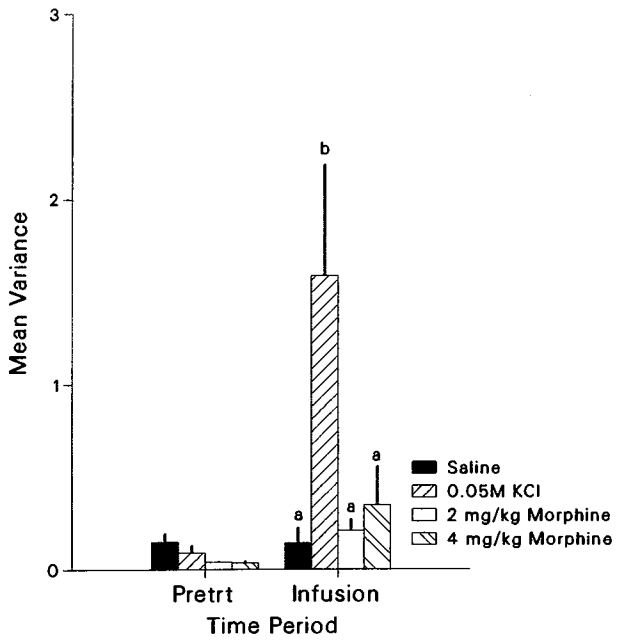

Fig. 6. Mean output variance of strain gauge output for experiment 3. Time period refers to pretreatment (Pretrt) and infusion periods (Infusion). Bars with different letters differ ($P < 0.05$).

treatment groups was observed during the pretreatment period. A significant treatment response was noted for rats given 0.05 *M* KCl when compared to controls. Rats pretreated with morphine showed no response to subsequent intravenous infusion of an irritating material. Both morphine-treated groups showed no significant difference from controls during the treatment period, and the response of both groups was significantly lower than that of KCl-treated rats. These results demonstrate that the administration of morphine ablates or reduces the sensation responsible for increased activity following intravenous administration of KCl.

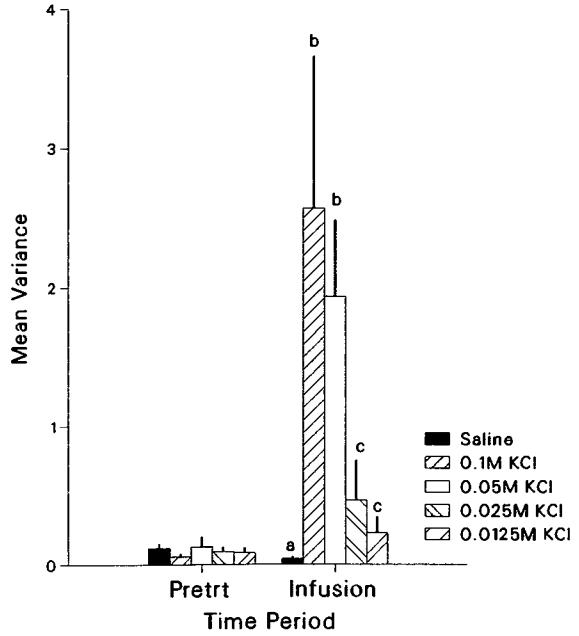

Fig. 5. Mean output variance of strain gauge output for experiment 2. Time period refers to pretreatment (Pretrt) and infusion periods (Infusion). Bars with different letters differ ($P < 0.05$).

The hypothesized mechanism by which the materials used in this study produce pain involve chemoreceptors or pain receptors located in the vessel wall. Sensory nerve fibers have been identified in blood vessels in the hindlimb of the rat, guinea pig, and cat (4), although specific pain or chemoreceptors in blood vessels have not been identified. Evidence of the presence of such receptors in blood vessels is indicated by the ability of intraarterial injection of chemical agents to evoke manifestations of pain (vocalization, struggling, hyperpnea, and hypertension) in animals (5). In addition, intravenous injection of a variety of substances causes pain, thrombosis, and thrombophlebitis in man (6,7). The mechanism by which intravenous administration of test solutions causes pain has not been identified. It has been hypothesized that high or low pH or osmolality or excitation of chemoreceptors or other pain receptors by the infusate is responsible for the pain reaction observed. It is evident that additional study into the innervation of peripheral veins is necessary to elucidate the mechanism of pain perception following intravenous administration of irritating materials.

In conclusion, the capability of the model to measure objectively the pain response to intravenously administered substances was demonstrated. This model would be most useful, and best applied, in the study of possible adverse effects of new compounds and/or vehicles early in the drug development process. Results of this screen show that the methodology is able to produce objective measurements and successfully demonstrate reaction to intravenous injection of substances known to produce clinical signs of pain. The

method is inexpensive, uses materials that are readily available in many labs, and requires little time.

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