Quantal Responses in the Randall–Selitto Assay (35615)

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The reaction threshold of the inflamed rat paw to pressure may be used to evaluate analgetic drugs (1). The analgetic component of activity of anti-inflammatory compounds may be demonstrated by a modification (2) to the method described by Randall and Selitto (1). Measured responses are recorded in these assays (usually in mm of Hg) even though cutoff pressures are necessarily specified. Other analgetic assays have been designed to yield quantal data (3, 4) and such data may be analyzed graphically by a probit transformation. By designing the Randall–Selitto assay to obtain quantal data, the determination of relative analgetic potencies of drugs is facilitated and the need for including cutoff pressures in the calculation of mean responses is obviated.

Materials and Methods. Reaction thresholds to pressure were determined with an "Analgesy-Meter" (Ugo Basile, Milan, Italy), a device exerting a force which increases at a constant rate (32 g/sec in the experiments reported) and which is continuously monitored by an indicator moving along a linear scale. The rat paw is positioned on a Teflon platform and the force is applied to the volar surface via a blunt Teflon cone. Male rats (Simonsen strain, Sprague-Dawley derived), weighing 110–125 g, were fasted overnight. Rats were selected for paw sensitivity by excluding those not responding to a force of 200 g for either foot. A maximum of 3 trials/paw was employed in this initial selection process. These trials also served as a "training" session to determine if the desired behavioral pattern of either a coordinated struggle or a withdrawal of the paw was present. Edema was induced by the injection of 0.1 ml of a continuously stirred 20% brewer's yeast suspension into the plantar surface of the left hind paw of each rat. Two hr later the rats received drugs by gastric intubation. Drugs were suspended in 4% acacia and the concentrations were adjusted such that each rat received 0.5 ml of suspension/100 g of body weight. Control rats received a like volume of the vehicle. Twenty rats served as the vehicle-treated controls and each dose of drug was administered to 10 rats. Eighty rats were handled conveniently, yielding three point dose–response curves for two drugs for each assay. Thirty min after dosing, reaction thresholds to pressure of the inflamed and noninflamed paws of the control group were recorded. The control group mean thresholds of the inflamed (yeast-injected) and noninflamed (uninjected) hind paws were determined. Rats in the drug-treated groups were designated as "protected" if the individual reaction thresholds to pressure exceeded the control group mean threshold by two standard deviations of that mean. To partially compensate for differences among drugs in the time after administration until maximum effect, reaction thresholds to pressure were determined at two time intervals after drug administration (30 and 60 min). Individuals in each group were scored as protected if their reaction threshold to pressure exceeded the control mean by two standard deviations at either time interval. The forces required to elicit reaction thresholds were not recorded in the drug-treated groups since the force was removed from the paw of the rats which were protected as soon as this force reached the predetermined control group mean threshold plus two standard deviations of that mean. This served to minimize tissue damage which might influence the second (60 min) reading. Trivial differences were found between the reaction threshold obtained at 30 and 60 min for the control group in preliminary experiments and the mean obtained for the control group at 30 min was chosen as the reference

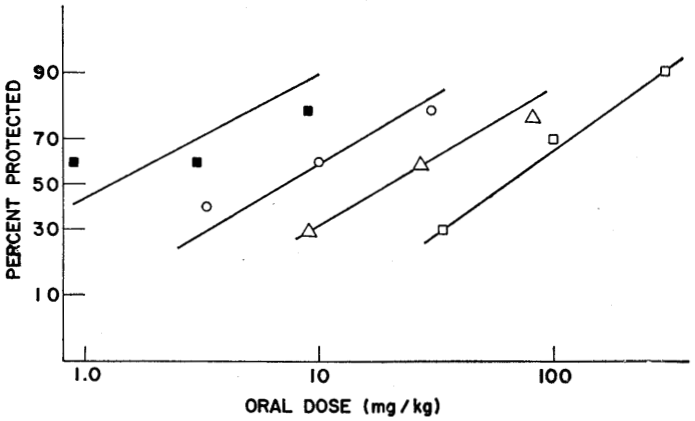

FIG. 1. Effect of analgetic compounds on the reaction threshold to pressure of the inflamed rat paw: indomethacin (■); diflumidone sodium (○); propoxyphene·HCl (△); and mefenamic acid (□).

threshold. The control group was scored as the drug-treated groups at the 60-min interval in subsequent assays. ED_{50} and relative potencies were determined by the method of Litchfield and Wilcoxon (5).

Results and Discussion. The percentage of control (vehicle-treated) rats protected for the 11 consecutive assays was 6.9% (inflamed paw) and 5.0% (noninflamed paw).

Morphine sulfate, 3 mg/kg sc as the salt, was included as a positive control in 9 assays. The reaction threshold to pressure was determined at 15 and 30 min after administration by this route. The percentage of paws of rats protected with this dose of morphine sulfate ranged from 40 to 90 for the inflamed, and 20 to 70 for the noninflamed paw, respectively. In a separate four point assay, the sc ED_{50} and 95% confidence limits for morphine sulfate were estimated to be 1.6 (0.9–3.0) mg/kg for the inflamed, and 2.4 (1.4–3.8) mg/kg for the noninflamed paw, respectively.

The regression lines obtained for four drugs are shown in Fig. 1. For clarity of presentation, the lines obtained for aspirin and phenylbutazone are not shown. The potency ratios and their 95% confidence limits for aspirin, phenylbutazone, mefenamic acid, propoxyphene HCl, diflumidone sodium, and indomethacin were estimated to be 1.0; 1.1 (0.4–2.8); 1.2 (0.5–3.3); 3.9 (1.5–10.5); 10.7 (3.6–31.5); and 56.3 (17.9–177), respectively. Diflumidone sodium, the sodium salt of 3-benzoyldifluoromethanesulfonanilide, is a new nonsteroidal compound which shows anti-inflammatory activity in standard assays for this type of compound (6). Since all drugs were not assayed simultaneously, the criteria of comparative bioassay are not met and the estimates of relative potencies are only approximations. The potency estimates obtained, however, agree reasonably well with published data for some of these drugs (1, 2, 7). The six regression lines showed homogeneity of fit and all slopes were parallel. The poorest fitting line was obtained for indomethacin but this would appear to be due to the selection of excessively high doses for the assay. Of the six drugs examined, only propoxyphene HCl afforded significant protection to the noninflamed paw (ED_{50} = 30 mg/kg). Elevation of the reaction threshold to pressure of the noninflamed paw in this assay is a characteristic of centrally acting analgetics (1).

Summary. The Randall–Selitto assay, measuring the reaction threshold of the inflamed rat paw to pressure, was modified to obtain quantal data. Inclusion of maximum "cutoff pressures" in the calculation of mean responses is not necessary and relative analgetic potencies of drugs may be determined graphically by a probit plot.

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