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Simple Methodology of Assessment of Analgesics' Addictive Potential in Mice

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PCHELINTSEV, M. V., E. N. GORBACHEVA AND E. E. ZVARTAU. *Simple methodology of assessment of analgesics' addictive potential in mice.* PHARMACOL BIOCHEM BEHAV **39**(4) 873–876, 1991.—Comparative investigation of analgesic (writhing test) and reinforcing (conditioned place preference) effects of 11 opioids over a wide range of doses was performed in mice. On the basis of the dose-response curves analysis, parameters of potency and efficacy of the reinforcing effect were determined. In one group of analgesics (etorphine, fentanyl, buprenorphine, morphine, promedol) ED_{50} in both tests were approximately equal. The other compounds (pentazocine, nalbuphine, nalorphine, butorphanol, U50,488H) demonstrated dissociation of two effects. The new indices (addictive index and safety index) for the prediction of analgesics' as well as other psychotropic drugs' abuse potential at the preclinical stage of their study have been suggested.

Conditioned place preference Analgesia Analgesics Addictive potential Preclinical study

A reliable determination of the addictive potential of new analgesics at the preclinical stage of study is of extreme importance for the assessment of their clinical and social safety. Intravenous self-administration in monkeys, dogs and rats proves to be the most commonly used experimental paradigm employed for this purpose (9). The model is based on reinforcing effects of morphine-like analgesics. The expert preclinical system using tests assessing psychoactive substance action on brain systems of "reward" and "punishment" has been developed recently in our laboratory (6,7). However, the methods mentioned above have certain drawbacks concerned with their technical complexity, need for pretraining of animals, surgery, duration of experiments and expense. Therefore, they haven't been considered to satisfy the demands of pharmacological screening procedures. In addition, these techniques do not allow the obtainment of simple and integrated indices of abuse liability that are convenient for the comparison of individual drugs.

Over the past years, the conditioned place preference paradigm in rats has been widely used (1,8). This technique has some advantages: the influence of a substance on instrumental reactions is eliminated, it is possible to test the reinforcing effect in a drug-free state, and a positive effect may be revealed sometimes even after a single administration. The method makes it possible to assess the effect of intracerebral injections and, in addition, to detect both positive and aversive drug influences.

This article presents results of studies using the conditioned place preference paradigm with special attention to a) use of mice, experimental animals convenient for screening procedures, b) investigation of dose dependency of reinforcing effect of opioids, and c) calculation of integral indices, similar to a classical therapeutic index, to characterize drugs' abuse liabilities.

METHOD

Male mice weighing 18–28 g were used in experiments. The animals were housed 6 per cage with granulated food and water ad lib.

The analgesic effects of the drugs were assessed using a writhing test based on chemical irritation produced by IP injection of 2% solution of acetic acid. The number of writhes per mouse during a 20-min period was calculated. The drugs were administered SC 30 min prior to the test. No more than three writhing reactions were considered to be the criterion of analgesia. The number of animals in a group with analgesic effects was determined. The quantal dose-effect relation for every drug was estimated using 4–6 doses. Every dose was tested in separate groups of 6–24 mice.

Place preference experiments were performed in plastic shuttle boxes. Guillotine doors (9 × 10 cm) separated every box into two equal-size (25 × 25 × 30 cm^3) compartments supplied with infrared movement detectors. The compartments differed in brightness, color (80 and 8 lux for white and dark brown, respectively) and floor texture (metal net in a light compartment and smooth floor in a dark one). The whole system consisted of 12 boxes connected to a processor for measuring time spent in the drug-paired side. Groups of 8–32 mice per dose were tested to reveal dose dependency of the effect.

The experiment involved three phases: first testing, conditioning, and second testing. The first phase (3 days) included two sessions of "adaptive" free investigation of both compartments during 30 min (the door between compartments was opened). On the third day, a control preexposure test was performed.

Only animals preferring the dark compartment, i.e., spending

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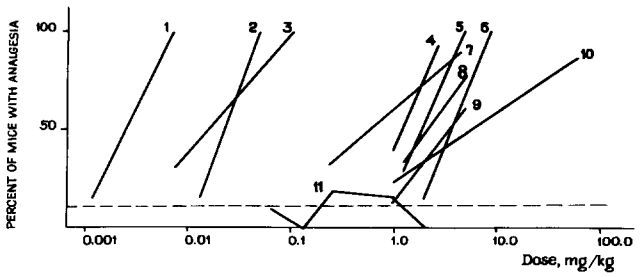

FIG. 1. Quantal dose-effect regression lines in writhing test. Drugs: 1—etorphine; 2—fentanyl; 3—buprenorphine (Temgesic); 4—U50,488H; 5—morphine; 6—promedol; 7—butorphanol (Moradol); 8—nalbuphine (Nubain); 9—nalorphine; 10—pentazocine (Lexir); 11—diprenorphine. Horizontal dotted line is the upper border of the saline effect.

more than 50% (15 min) of the whole test time there, were chosen for further studies.

The conditioning phase consisted of eight 30-min sessions. The animals were confined to one compartment of the shuttle box with the guillotine door closed. During four of the conditioning sessions, the mouse was pretreated SC with vehicle and placed into the black compartment. In the remaining four sessions, the animal received the drug treatment SC and was confined to the initially unpreferred white compartment. The alternate vehicle and drug pairings occurred daily during 4 days.

The second test was carried out 48 hours after the last conditioning session. The animals were placed in the shuttle box with doors opened for 30 min. The time spent in drug-paired compartments was recorded, and the shift of this time in postconditioning versus preconditioning periods served as the gradual preference score. Also, the number of animals spending more than 50% of the time in the drug-conditioned side was calculated for every dose of drugs (quantal preference score).

Potencies of drugs in writhing and CPP tests were estimated by the quantal dose-effect curves analysis (Litchfield-Wilcoxon method) with median effective doses (ED_{50}) calculation. Significance of time in drug-paired side changes was estimated using Student *t*-tests and Mann-Whitney tests.

The following drugs were used: morphine, nalorphine, promedol (synthetic meperidine-like drug), fentanyl (commercial formulations, USSR), pentazocine (Lexir, Gedeon Richter), etorphine, diprenorphine (The Institute of Organic Elements Compounds, Moscow), buprenorphine (Temgesic, Reckitt A. Colman), nalbuphine (Nubain, Du Pont de Nemours), butorphanol (Moradol, Galenica), and U50,488H (gift of Upjohn Co.).

RESULTS

Figure 1 shows the relation between the dose of drugs studied and their antinociceptive effect in the writhing test. Etorphine, buprenorphine and fentanyl exhibited analgesic action in the range of doses from one thousandth to one hundredth mg/kg (Table 1). The regression lines for other drugs were distributed

TABLE 1

ED_{50} OF ANALGESICS IN WRITHING AND PLACE PREFERENCE TESTS AND INDICES DERIVED FROM THEIR COMPARISON

Substance (drug)	ED_{50} (confidence limits) mg/kg in Tests: Analgesia (A)	Reinforcing Properties (B)	Addictive Index (A/B)	Safety Index (B/A)
Etorphine	0.0023 (0.002–0.003)	0.0036 (0.002–0.007)	0.64	1.56
Buprenorphine	0.016 (0.009–0.027)	0.027 (0.012–0.062)	0.59	1.69
Fentanyl	0.019 (0.01–0.03)	0.019 (0.008–0.045)	1.0	1.0
Morphine	1.95 (1.25–3.07)	3.78 (2.21–6.48)	0.51	1.94
Promedol	3.94 (2.86–5.42)	6.84 (2.85–16.4)	0.58	1.74
Nalorphine	3.23 (2.0–5.1)	17.5 (8.7–34.7)	0.18	5.42
Pentazocine	3.59 (1.9–6.3)	20.6 (12.8–32.3)	0.17	5.74
Butorphanol	0.54 (0.36–0.83)	†	—	—
Nalbuphine	2.10 (1.25–3.70)	†	—	—
U50,488H	1.60	*	0	Infinite
Diprenorphine	†	*	0	Infinite

*No effect.
†Unconstant effect, no dose-dependency.

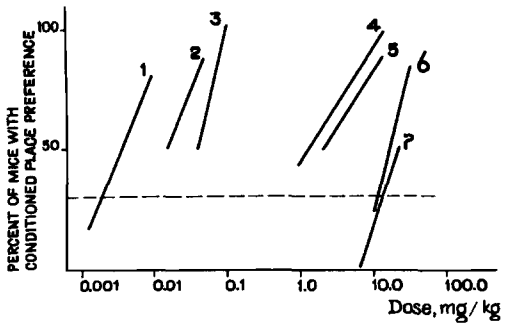

FIG. 2. Quantal dose-effect regression lines in conditioned place preference test. Drugs: 1—etorphine; 2—fentanyl; 3—buprenorphine (Temgesic); 4—morphine; 5—promedol; 6—pentazocine (Lexir); 7—nalorphine. Horizontal dotted line is the upper border of the saline effect.

within the range from one to ten mg/kg. Diprenorphine did not produce any notable analgesic action in this model and did not demonstrate a dose-effect relation.

Figures 2 and 3 illustrate the results of conditioned place preference studies. The drugs of the first group (etorphine, fentanyl, buprenorphine, morphine, promedol, pentazocine, and nalorphine—Fig. 2) demonstrated dose-dependent reinforcing effects, while drugs of the second group (Fig. 3) either did not produce any reinforcing action (diprenorphine, U50,488H) or failed to exhibit any dose-dependent action (butorphanol, nalbuphine).

According to their potencies, the drugs from the first group are concentrated in three clusters: 1) drugs of high potency (etorphine, fentanyl, buprenorphine), with ED_{50}s within the microgram range (Table 1); 2) substances with the lowest potencies (pentazocine, nalorphhine), with ED_{50}s exceeding 10 mg/kg; and 3) intermediate-potency substances (morphine, promedol), with ED_{50}s within the range of 1–10 mg/kg. Thus the potencies of the substances mentioned above had more than a 1000-fold difference.

In experiments with buprenorphine and nalorphine, the dose-response curves had the U-inversed form (not shown in Fig. 2) with the maximum points at the following doses: buprenorphine—0.1 mg/kg (90–100%), nalorphine—20 mg/kg (50%). The increase of dose eliminated (nalorphine) or diminished (buprenorphine) the reinforcing effect.

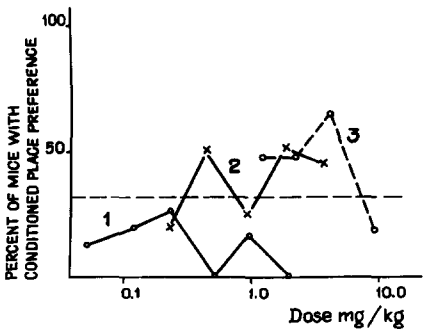

FIG. 3. Dependence between the dose of the substances studied and the reinforcing effect (conditioned place preference test). Drugs: 1—diprenorphine; 2—butorphanol (Moradol); 3—nalbuphine (Nubain). The effect of U50,488H isn't shown in the figure. Horizontal dotted line is the upper border of the saline effect.

TABLE 2

MAXIMAL INCREASE OF TIME SPENT IN DRUG-PAIRED COMPARTMENT AFTER CONDITIONING

Drug	Dose (mg/kg)	Increase of Time Spent in Drug-Paired Compartment (s, mean ± s.e.m.)
Etorphine	0.008	312.8 ± 76.9†
Fentanyl	0.05	376.1 ± 73.8†
Morphine	20.0	498.5 ± 114.4†
Promedol	20.0	337.8 ± 96.1†
Nalbuphine	5.0	168.6 ± 91.4*
Butorphanol	0.5	142.5 ± 66.9*

*$p<0.05$ (Mann-Whitney test).
†$p<0.01$ (Student t-test). Differs significantly from saline.

The number of mice with conditioned place preference in groups receiving various doses of diprenorphine and U50.488H did not differ significantly from the control group. Nalbuphine and butorphanol failed to produce a constant secondary reinforcing effect (Fig. 3). Only the 0.5 mg/kg (butorphanol) and 5 mg/kg (nalbuphine) caused the statistically significant effect.

In Table 2, the intensity of the reinforcing effects of drugs is compared using maximal values of the increase in the time spent in the drug-paired compartment. The table shows that the drugs differ in their reinforcing efficacies (e.g., maximal effect of nalbuphine and butorphanol was less compared with the other analgesics investigated).

DISCUSSION

Present results support well-known data showing that the writhing test is a sensitive method for detecting the antinociceptive actions of pharmacological substances (2,10). The reinforcing effects of agonists in the conditioned place preference paradigm are also amply covered [see (1, 8, 11)]. The novelty of this study appears to be the comparative investigation of these effects of analgesics in mice, with special attention to dose dependency. An attempt has been made to measure potency and efficacy of the reinforcing effect using routine pharmacological indices (ED_{50} and maximal effect).

Mean effective doses of the drugs studied in both tests are presented in Table 1. Several compounds (etorphine, buprenorphine, fentanyl, morphine, and promedol) are seen to demonstrate similarity of potencies in both tests. Other substances (nalorphine, pentazocine, butorphanol, nalbuphine, and U50,488H) do not exhibit the correlation of these effects. They were more active in the writhing test in comparison to conditioned place preference. The mean effective reinforcing doses of nalorphine and pentazocine were 5–6 times higher than the analgesic ones. Nalbuphine, butorphanol and U50,488H were characterized by either inconstant or nonsignificant reinforcing actions.

Etorphine, buprenorphine, fentanyl, morphine and promedol act as "full" agonists, as they produce the maximal effect (80–100% of mice with preference of drug-paired side), whereas pentazocine, nalorphine, butorphanol, and nalbuphine demonstrated "partial" agonistic properties.

U50,488H is known to be a selective agonist of kappa receptors, and this compound has no reinforcing effect. Many agonist-antagonist analgesics are also known to interact somewhat

with this subtype of receptors (3). On the other hand, morphine, fentanyl, and etorphine are prototypic mu agonists and reinforcing substances. It evidences the participation of mu receptors in mediation of opioid reward. It is of interest that pentazocine and nalorphine, known as mixed agonist-antagonists, behave as partial mu agonists in the model of conditioned place preference. In investigations of the discriminative stimulus properties in mice (N. A. Patkina, unpublished data), pentazocine, butorphanol, and buprenorphine were shown to possess stimulus properties similar to morphine. Comparative analysis of the role of opioid receptors subtypes in analgesic and reinforcing effects was performed by us elsewhere (2, 4, 5).

Slopes of quantal dose-response curves for analgesia and drug reward for substances demonstrating dose dependency in both experimental paradigms were not significantly different. It allows the comparison of parameters of potencies of drugs and calculation of integral indices (Table 1). These indices show how many times higher are reinforcing (adverse effect) ED_{50}s compared to analgesic (therapeutic effect) ED_{50}s (safety index), or, vice versa, what part of reinforcing (unwanted effect) ED_{50} is therapeutic (analgesic) ED_{50} (addictive index). Also, the "ceiling" of the reinforcing effect might give additional information on the efficacy of the drug reward effect.

Therefore, the methodology proposed gives the opportunity to characterize analgesics' addiction potential on the basis of simple, efficient and economic tests in mice. This approach, based on the comparative dose-response analysis of reinforcing (unwanted) and therapeutic effects, can be extended to other groups of neuropsychotropic drugs in predicting and investigating their abuse liabilities.

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