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Continuous Infusion in Rats as a Method of Assessing Morphine-Like Physical Dependence for Opiates

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McCARTHY, P. S., G. METCALF AND S. J. HOWE. *Continuous infusion in rats as a method of assessing norphine-like physical dependence for opiates.* PHARMAC. BIOCHEM. BEHAV. 16(5) 725–729, 1982.—Continuous intraperitoneal infusion of opiates into rats has been investigated as a method of predicting the physical dependence liability of such drugs in humans. The method differs from and has several advantages over previous methods. Using the syndrome of abstinence behaviour precipitated by naloxone for a number of representative narcotics it was possible to rank order these drugs for their ability to produce physical dependence in human subjects. The method has been used to evaluate the dependence liability of a number of potent analogues of enkephalin.

Opiate-dependence Morphine Narcotics Enkephalins

THE recent discovery and synthesis of opioid peptides has given a major impetus to research relating to mechanisms of analgesia and their relationship to physical dependence.

As part of this effort to attain non-addictive opioids there has been considerable interest in methods to assess the addictive liability of new opioid molecules in animals [16] and man [12]. The dependence producing properties of opioids have been studied in a number of species and it has become apparent that important species differences exist to the effect of morphine and that different species respond to various chemical classes of opioid in different ways. Consequently it is difficult to decide which species can best serve as a predictive model for man.

The present study was undertaken to investigate the ability of a variety of opioid drugs to induce physical dependence in the rat using a technique of continuous intraperitoneal infusion [17]. The results obtained have been compared to assessment of addictive liability made in human subjects in order that the predictive values of the rat model may be ascertained.

METHOD

Animals

Male Sprague-Dawley rats 140–160 g initial weight were used throughout.

Implantation of Infusion Cannulae

The technique was essentially that described by Teiger [17]. Briefly rats were anaesthetised with 1% halothane and intraperitoneal polythene cannulae (PP 50) were implanted using a trochar. Each cannulae was directed via the subcutaneous tissue to an exit point at the back of the neck. Individual animal harnesses were fitted to protect this exit point and each cannulae was connected via a liquid swivel joint to a continuous infusion pump. Animals were individually caged and allowed free access to both food (Labsure diet) and water. Following surgery animals were allowed to recover for 3–4 days before being subjected to drug infusions. The infusion rate was 6 ml/24 hr. Post mortem examinations at the end of experiments showed no evidence of peritoneal irritation or infection as a result of the cannulae implantations.

Development of Dependence

Direct dependence was allowed to develop over a 48 hr period of infusion at constant dosage. At the end of 48 hr the infusion was terminated and the animals removed from their harnesses. After a further period of 30 min the animals were weighed and abstinence was precipitated by a single injection of naloxone (3 mg/kg SC). The rats were placed in a rectangular plastic bowl (35×45×15 cm) and behavioural signs of abstinence were observed for the following 10 min. Loss of body weight was assessed after a period of 3 hr.

Assessment of Abstinence

The morphine abstinence syndrome varies with different

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TABLE 1

ABSTINENCE SIGNS, ASSESSED ON A QUANTAL BASIS, PRECIPITATED BY NALOXONE (3 mg/kg, SC) AFTER INFUSIONS OF MORPHINE

Morphine Infusion schedule mg/kg/24 hr	n	% Animals exhibiting each sign: Weight loss >2 g*	WDS >2	Escape attempts	Writhing episodes	Teeth chatter	Diarrhoea	Gland sec.	Ptosis	Penile stim.	Ear blanch	Mean behavioural score
Saline	7	0 (1.4 ± 0.2)	0	0	0	0	0	0	0	43	0	4.3
1	7	14 (1.5 ± 0.5)	14	28	0	0	0	0	0	0	0	5.6
3	7	57 (3.1 ± 0.4)	43	0	43	14	57	0	0	29	0	24.3
6	7	43 (2.6 ± 0.2)	43	0	14	0	57	0	0	29	0	18.6
12.5	7	86 (3.5 ± 0.4)	43	43	57	14	100	0	0	14	0	35.7
25	8	100 (5.0 ± 0.4)	100	50	50	100	100	0	0	100	0	60.0
50	7	100 (6.5 ± 0.4)	86	43	57	100	100	14	29	100	71	70.0
100	8	100 (7.1 ± 0.1)	38	88	13	100	100	88	13	100	100	74.0

WDS=wet dog shakes; gland sec.=glandular secretions, i.e., lachrymation, salivation or rhinorrhoea; penile stim.=penile licking and seminal emission; ear blanch=ear blanching.

*Figures in parentheses represent mean % loss body weight ±S.E.M. measured 3 hr after naloxone injection.

species but in each case it is expressed by a variety of behavioural and autonomic effects. Some of these effects may be measured whilst others are merely apparent or non-apparent (i.e., quantal effects) in individual animals. In these experiments the following effects were measured (non "blind") during the 10 min after naloxone injection—number of escape attempts, number of shaking episodes (wet dog shakes) and number of writhing episodes. In addition the following effects were observed on a quantal, all or nothing, basis—teeth chattering, diarhoea, ptosis, glandular secretion (lachyrimation, rhinorrhoea or salivation), penile stimulation (erection and licking), ear blanching. Loss of body weight (expressed as % body weight loss) was measured over a 3 hr period after naloxone administration.

Subsequently in an attempt to obtain a single index representative of the whole syndrome the measured signs were also caluclated on a quantal basis (i.e., number of animals exhibiting >2.0 g weight loss, number of animals exhibiting >2 episodes of wet dog shaking, number of animals exhibiting any escape attempts, or number of animals exhibiting any writhing episodes). Thus all 10 types of behaviour observed were represented as a quantal score representing the % animals exhibiting each sympton. The mean % incidence (mean behavioural score) was calculated as an index of the whole abstinence syndrome.

Assessment of Analgesic Activity

The analgesic potencies of enkephalin analogues were assessed using the hot water rat tail flick test [11] and the mouse writhing test [10].

Drugs Used

Naloxone hydrochloride (Endo.), morphine sulphate (Macfarlan-Smith), pentazocine base, cyclazocine base (Sterling Winthrop), codeine phosphate (Macfarlan-Smith) nalorphine (Wellcome), propiram (Dr. Hoffmeister, Bayer), propoxyphene (Macfarlan-Smith), butorphanol tartrate (Bristol Laboratories), enkephalin analogues were supplied by Dr. B. A. Morgan (Reckitt and Colman). In all cases doses were calculated as the base.

RESULTS

Direct Dependence Studies with Morphine

Groups of animals were exposed to morphine using a variety of dosage schedules ranging from 1–100 mg/kg/24 hr. Animals exposed to doses greater than 12.5 mg/kg/24 hr. exhibited behavioural effects soon after the infusion began that were consistent with acute administration of opiates (i.e., hyperactivity, grooming, gnawing, exopthalmos), but tolerance to such effects was marked as the infusions continued.

After 48 hr the degree of physical dependence produced by the various doses of morphine was estimated by assessing ten symptoms (see Methods) of withdrawal precipitated by an injection of naloxone (3 mg/kg, SC). The degree of weight loss (expressed at % loss body weight) during the 3 hr period after naloxone administration exhibited a linear relationship to the log dose morphine administered ($r=0.96$; $y=2.8x+1.2$; $p<0.001$). Similarly when considered as a quantal effect of the number of animals exhibiting a weight loss of more than 2 g, the weight loss effect was related to dose

TABLE 2

ABSTINENCE SIGNS, ASSESSED ON A QUANTAL BASIS, PRECIPITATED BY NALOXONE (3 mg/kg, SC) AFTER 48 HR INFUSIONS OF 100 mg/kg/24 HR OF VARIOUS OPIOID DRUGS IN RATS COMPARED TO PEAK HIMMELSBACH SCORES IN HUMAN SUBJECTS

		% Animals exhibiting each symptom											
Drug	n	Weight Loss >2 g*	WDS >2	Escape Attempts	Writhing Episodes	Teeth Chatter	Diarrhoea	Gland sec.	Ptosis	Penile stim.	Ear blanch	Mean behavioural score	Human Score†
Saline	7	0 (1.4 ± 0.2)	0	0	0	0	0	0	0	43	0	4.3	—
Morphine	8	100 (7.1 ± 0.1)	38	88	13	100	100	88	13	100	100	74.0	36.8
Codeine	9	100 (6.8 ± 0.9)	100	30	60	80	70	0	0	20	0	46.0	26.0
Cyclazocine	8	25 (2.1 ± 0.5)	50	12	62	63	25	0	0	13	0	25.0	18.8
Pentazocine	5	80 (4.0 ± 0.5)	40	40	0	100	60	0	0	0	60	38.0	15.8
Nalorphine	9	33 (3.0 ± 0.3)	77	11	66	33	22	0	0	0	0	24.2	18.2
Propiram	10	90 (5.4 ± 0.6)	70	0	30	10	60	0	0	10	0	27.0	24.3
Propoxyphene	12	100 (6.3 ± 0.4)	75	50	75	58	100	0	0	17	42	51.7	29.5
Butorphanol	10	100 (6.2 ± 0.7)	70	40	40	50	40	0	0	20	0	36.0	26.3

*Figures in parentheses represent mean % body weight ± SEM measured 3 hr after naloxone injection.
†Peak Himmelsbach Scores. Data from refs [7, 12, 13]. Other abbreviations as in Table 1.

(Table 1). The number of animals making escape attempts also appeared linearly related to dose whereas the relationship between the number of animals exhibiting wet dog shakes or writhing movements exhibited a biphasic relationship to the dose of morphine administered (Table 1).

Consideration of the other abstinence signs assessed only on a quantal, all or none, basis (i.e., teeth chattering, diarrhoea, ptosis, penile stimulation, glandular secretions, ear blanching; see Table 1) showed that, with the possible exception of ptosis, the incidence of such signs appeared to be related to the dose of morphine infused. Both teeth chattering and diarrhoea appeared to be sensitive indices of dependence in that some animals exhibit these signs after an infusion of only 3 mg/kg/24 hr morphine. On the other hand glandular discharges (salivation, lachrymation or rhinorrhoea), ptosis or ear blanching were only provoked after infusion of high doses of morphine. Taking all ten symptons into consideration when assessed on a quantal basis there was a linear relationship between the mean behavioural score and the dose of morphine administered ($r=0.96$; $y=37.1x+1.7$; $p<0.001$).

Comparison with limited data available as Himmelsbach scores from human studies on morphine dependence [12] suggests that a similar relationship exists in rats and humans between the severity of the abstinence syndrome observed and the dose of morphine used to produce dependence.

Predictive Value of the Rat Infusion Model

To assess the predictive value of the rat infusion model a number of opiate drugs which have been evaluated in human subjects [15] were tested. All the drugs were infused at 100 mg/kg/24 hr over a period of 48 hr. At the end of this period abstinence was precipitated by naloxone (3 mg/kg, SC) and assessed as described in Method section.

The results obtained are set out in Table 2. Examination of the table shows that there is considerable variation in the number of animals exhibiting each sympton.

Quantitative comparison between the results produced by opiate drugs in rats with the effects produced by the same drugs in human addicts was difficult because of the different dosage regimes, different routes of drug administration and the different methods of assessment. However, using the nonparametric Spearman Rank Correlation Test it was possible to demonstrate a correlation between the % weight loss in rats and the peak Himmelsbach scores in human addicts ($r_s=0.83$; $p<0.01$; $n=8$) and a similar correlation between the mean rat behavioural score and the Himmelsbach scores ($r_s=0.72$; $p<0.05$; $n=8$).

Dependence Liability for Enkephalin Analogues

Since the determination of the structure for enkephalin, a number of analogues have been synthesised which are comparable to morphine with respect to potency in antinociceptive tests and are also resistant to enzymatic degradation. A selection of such analogues were examined for dependence liability using the rat infusion model.

Initial experiments determined that an infusion rate of 100 mg/kg/24 hr induced toxic effects (catalepsy, respiratory depression, coma and death). Infusion at the lower rate of 30 mg/kg/24 hr produced acute behavioural effects consistent

TABLE 3

PROFILES OF OPIOID ACTIVITY (ANTINOCICEPTIVE TESTS AND PRECIPITATED ABSTINENCE) FOR A SELECTION OF ENKEPHALIN ANALOGUES

Analogue	Amino acid sequence	ED_{50} mg/kg, IV*		Dependence model†	
		MWT	RTF	% weight loss	Mean behavioural score
A	MeTyr-DAla-Gly-MePhe-NH $(CH_2)_3S(O)$ Me	0.004	0.3	7.2 ± 1.4	50.0
B	Tyr-DAla-Gly-MePhe-NH $(CH_2)_3$ N Me_2	0.28	2.1	4.2 ± 0.6	28.6
C	Tyr-DAla-Gly-MePhe-NH $(CH_2)_3$ N (O) Me_2	0.00003	1.85	7.9 ± 0.6	43.0
D	Tyr-DAla-Gly-MePhe-NH CH_2CH_2 N Me_2	0.003	1.4	3.6 ± 0.4	23.9
E	Tyr-DAla-Gly-MePhe-NH CH_2CH_2 N (O) Me_2	0.00000008	0.32	7.7 ± 0.9	28.8

*Antinociceptive ED_{50} values. MWT= mouse writhing test [10]. RTF=rat tail flick test [11].

†Enkephalins infused at 30 mg/kg/24 hr for 48 hr. Infusion was then stopped and abstinence provoked by 3 mg/kg, SC naloxone as described in Method section.

with opiate administration (e.g., hyperactivity and excessive grooming) although tolerance to such effects was marked. At the end of 48 hr abstinence was precipitated by naloxone (3 mg/kg SC) as described in Methods. As judged by loss of body weight and the behavioural effects produced, all of the enkephalin analogues produced some degree of physical dependence (Table 3). With the exception of analogue E, there was good agreement between the two indices of dependence (weight loss and mean behavioural score) for each compound. Analogue E exhibits marked behavioural effects some of which are non-opiate in character (e.g., the potent effect in the mouse writhing test only part of which is naloxone reversible; see [4]) and it would appear that such effects may diminish the behavioural index of dependence.

DISCUSSION

In the present study the use of continuous intraperitoneal infusion has been examined as a method of predicting the ability of narcotic analgesic drugs to induce physical dependence in man. Traditionally physical dependence liability for analgesic drugs has been assessed in laboratory animals after multiple injections [9] or the implantation of depot sources [6,18]. All of these methods involve problems associated with either the determination of the actual dose of narcotic absorbed or the maintenance of a constant level of exposure to the narcotic drug [2].

Various authors have investigated abstinence behaviour following morphine dependence and whilst there is broad agreement concerning the main items of behaviour observed (e.g., weight loss, teeth chattering, escape attempts, diarrhoea, wet dog shakes, salivation) there is also considerable variation concerning the appearance or non-appearance of other symptons (e.g., exopthalmos, chromodacryorrhea) and also in the degree of dependence which is necessary before any particular sympton is observed [1, 3, 5, 14, 19]. Although precipitated abstinence is a complex phenomenon with considerable inter-animal variation it seems probable that most of the variation apparent in the literature is due to the subjective methods of assessment used and to the wide variations in the bioavailability of morphine consequent on the diverse methods of administration. The present technique represents an attempt to overcome these problems of bioavailability.

Consideration of the behaviour observed during precipitated abstinence after morphine dependence allowed several conclusions. There was wide inter-animal variation in both the symptoms exhibited and their intensity (e.g., the incidence of penile stimulation in the control group was greater than in any other group except those treated with high doses of morphine). Only the % weight loss and the mean behavioural score obtained from all 10 symptons (thus "diluting" out a lot of individual variation) correlated with the dose of morphine infused. The observation that the intensity of some symptons (e.g., wet dog shakes, writhes and possibly ptosis) actually declined in animals treated with larger doses of morphine could help to explain some of the apparent contradictions in the literature as to whether certain withdrawal symptons are observed during precipitated abstinence. No attempt has been made to contrast the abstinence syndrome observed with analgesic potency as each drug will produce differing levels of analgesia in animals dependent upon route of administration, species, degree of partial agonism, test method and the nature of the interaction with the particular type of opiate receptor(s) involved.

Naloxone was used to precipitate abstinence after a variety of narcotic drugs chosen as representative of both pure agonists and mixed agonist/antagonists. Infusion of a fairly high dose of test drug enabled information to be gained concerning the potential dependence liability of the compound whilst identical doses needed to be infused in order to make drug comparisons in this respect. On this basis it would appear that certain drugs (e.g., cyclazocine and pentazocine) do not produce the same degree of physical dependence as morphine. The scores obtained (% weight loss and mean behavioural score) were used to rank order the analgesic drugs for dependence liability. There was a significant correlation coefficient between either of these rank orders and the rank order for the same drugs obtained using peak Himmelsbach Abstinence Scores in human addicts, thus suggesting that either of the indices from the rat infusion model could be used to predict relative dependence liability for new drugs in human subjects.

As a result of this finding the model was used to investigate the physical dependence liability of several analogues of methionine enkephalin. These analogues were known to be resistant to enzymatic degradation and comparable to mor-

phine with respect to antinociceptive potency in rats [4]. The results obtained showed that all five analogues induced varying degrees of physical dependence. The toxicity associated with infusions of 100 mg/kg/24 hr, which necessitated reducing the dose to 30 mg/kg/24 hr indicated the acute narcotic potential of these compounds. When compared with either normorphine or methionine enkephalin in the electrically stimulated mouse vas deferens preparation, all these analogues behaved in a morphine-like fashion ([8], and personal communication, Dr. C. F. C. Smith). With the exception of analogue E there was also good agreement between weight loss and behavioural score as indices of dependence (this was also true for the other drugs examined). Analogue E also exhibited potent activity in the less specific mouse writhing test and as at least some of this activity was not reversed by naloxone, so that it is possible that other CNS effects of this analogue are diminishing the behavioural expression of abstinence.

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