EVALUATION OF MORPHINE AND CHLORDIAZEPOXIDE WITHDRAWAL WITH TELEMETRY

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INTRODUCTION

There have been recent concerns from regulatory authorities that withdrawal effects may be a feature of many CNS acting substances. Assessment of withdrawal effects has therefore now become an important part of safety pharmacology and the procedures used need to be sensitive and widely applicable, i.e. not specific to any particular drug class or mechanism of action. Although several of the guidelines published by regulatory authorities have suggested using tests for anxiety and cognition, these procedures are often difficult to carry out repeatedly because of habituation and learning effects, i.e. the effects of the first evaluation interfere with interpretation of subsequent tests. This makes them difficult to apply to novel drug substances whose pharmacodynamics and type of effect are not known in advance. We have therefore concentrated on expanding the range of somatic measures which could be broadly described as objective measures of well-being, such as food-intake and body weight gain combined with telemetry to evaluate home-cage activity, as well as more physiological measures such as body temperature, mean blood pressure and heart rate.

In the present study we have validated this approach using morphine and chlordiazepoxide.

MATERIAL & METHODS

Groups of 8 male Wistar rats weighing approximately 260 g were anesthetized and given 7.5 mg/kg s.c. carprofen (Rimadyl™). Following a midline incision of the abdomen, a DSI TL11M2-C50-PXT implantable telemetric device was introduced into the peritoneal cavity and its catheter inserted upstream into the descending aorta, below the renal arteries. The abdominal and skin incisions were then closed and the animals were given 100 mg/kg amoxicillin i.m., which was repeated 24 hours later.

Approximately 1 week later, the animals were placed individually within their home cage located above a telemetry receiver (DSI) to record the following parameters: Mean, systolic and diastolic arterial blood pressure (mmHg); Heart rate (beats/min; derived from pulse blood pressure); Body temperature and Locomotor activity. Recordings were taken in blocks of 30 seconds every 5 minutes for periods of 24 hours starting two days before administration. Data are presented for the day preceding administration (Day 0) Day 1, Days 18, 19 and 20 of administration and then for an 8-day period of withdrawal.

All data were acquired and analyzed using the DSI software (Dataquest A.R.T. TM version 2.0 or 2.3).

In addition to the telemetry measurements, rats were weighed each day and food intake over the previous 24hrs (from Day 18 to Day 28) was measured.

A- Effects of morphine

Three treatment groups were studied: a) vehicle, b) morphine HCl 32 mg/kg b.i.d. and c) morphine HCl 64 mg/kg b.i.d. Each treatment was administered for 20 days (last treatment administered on the morning of the 20th day) followed by an 8-day withdrawal period during which rats received once daily vehicle injections.

B- Effects of chlordiazepoxide

Four treatment groups were studied: a) vehicle, b) chlordiazepoxide HCl 16 mg/kg b.i.d. c) chlordiazepoxide HCl 32 mg/kg b.i.d and d) chlordiazepoxide HCl 64 mg/kg b.i.d. Each treatment was administered for 20 days (last treatment administered on the morning of the 20th day) followed by an 8-day withdrawal period during which rats received once daily vehicle injections.

RESULTS

A- Effects of morphine

The results show that chronic morphine reduced food intake and body weight gain, as well as disrupting the normal circadian patterns of body temperature, motor activity, blood pressure and heart rate.

Repeated handling and vehicle injections had little impact on the measured parameters.

Following withdrawal, both groups of morphine-treated rats showed a marked decrease in food intake and loss of weight which were maximal on day 2 and 3 of withdrawal (i.e. Days 22 and 23), respectively.

There was also a marked nocturnal hypothermia (-2°C) and a moderate diurnal hypertension (+30 mmHg) on day 1 and 2 of withdrawal (i.e. Days 21 and 22), respectively. Activity and heart rate showed small diurnal increases over the first 3 days of withdrawal (i.e. Days 21 - 23). The intensity and duration of effects were broadly dose-related.

All parameters returned to normal within 8 days after withdrawal.

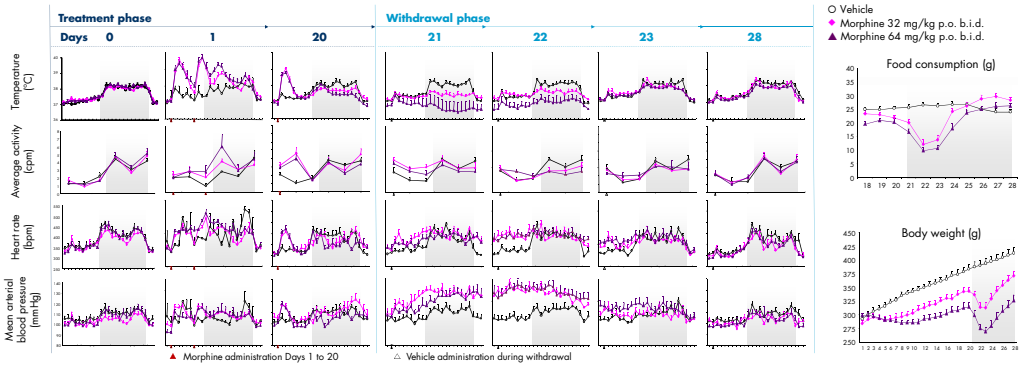
B- Effects of chlordiazepoxide

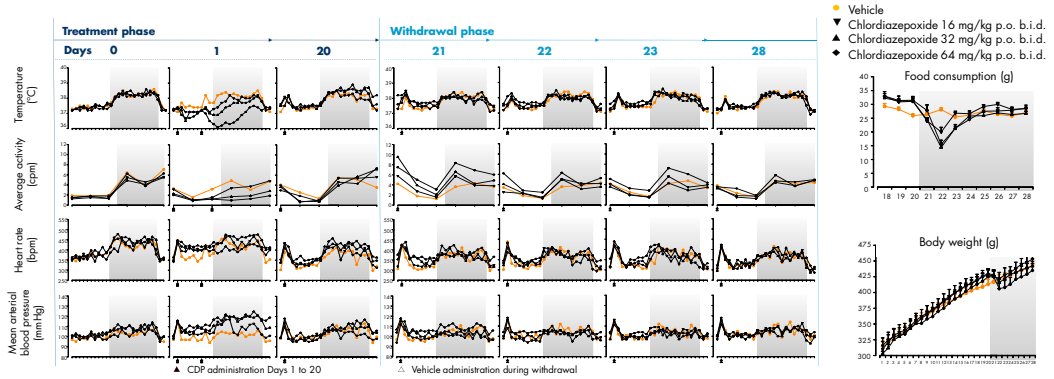
The results show that chronic chlordiazepoxide had only minor effects on food intake and body weight gain. During the first few days of administration there were marked and dose-related effects on body temperature, locomotor activity and blood pressure but all these effects tolerated out by the 10th day of administration.

Repeated handling and vehicle injections had little impact on the measured parameters.

Following withdrawal, all groups of chlordiazepoxide-treated rats showed a marked decrease in food intake and loss of weight. There was also a clear dose-related hyperactivity as well as more modest but dose-related diurnal increases in body temperature, blood pressure and heart rate. All effects were most marked on the day following withdrawal (i.e. between 24 and 48h after the final administration), but the effects on body temperature and locomotion were apparent at the end of the dark-phase less than 24h after the final administration.

All parameters returned to normal within 8 days after withdrawal.





Legend to figures: Each graph shows data for 24 hours starting from 9h00 (except average activity starting from 7h00: 4-hour blocks). Dark bar and zone indicate dark phase 19h00 to 7h00.

CONCLUSION

These data confirm and extend many of the changes previously reported but also show that telemetry can be used to further broaden the range of parameters measured during withdrawal. This may increase the sensitivity of the model to changes that might otherwise be missed. It is suggested that using somatic measures is a relatively simple way to identify treatments that may induce dysthymic and physiological signs of withdrawal that may reflect an animal feeling discomfort or of feeling unwell. The present method is applicable to a wide range of drug classes without a priori knowledge of the type or timing of their withdrawal effects. Once the time course of these effects has been identified in this way, supplementary tests such as those for cognition or anxiety may be carried out at adequate periods in separate studies.

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