

Effect of withdrawal of diazepam or morphine treatment on gastric motility (charcoal meal test) in mice: Possible role of different central and peripheral receptors

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Increased gastrointestinal motility in mice as one of the withdrawal symptoms of commonly abused drugs like diazepam or morphine and its possible mechanism of action was studied. Male Laka mice (20-25 g) were made addict to either diazepam (20 mg/kg, ip for 7 days) or morphine (10 mg/kg, sc for 9 days). Withdrawal symptoms were noted 24 hr after the last injection of diazepam or morphine. The animals were injected with Ro 15-1788 (flumazenil) (1 mg/kg, ip) or naloxone (2 mg/kg, ip) in the respective group to precipitate the withdrawal symptoms. Gastrointestinal motility was assessed by charcoal-meal test. Animals developed tolerance to acute sedative effect of diazepam, and similarly to the acute nociceptive action of morphine. On abrupt cessation of these drugs after chronic treatment the animals showed hyperlocomotion and hyperreactivity in diazepam withdrawal group and hyperalgesia on hot plate in morphine withdrawal groups, respectively. Increase in gastrointestinal motility was observed in all the drug withdrawal groups. Treatment with respective antagonists, Ro 15-1788 (flumazenil) and naloxone precipitated the withdrawal symptoms. The results suggest the involvement of both central and peripheral receptors of benzodiazepines and opioid (μ) receptors in the withdrawal symptoms of the benzodiazepines and morphine, respectively.

Keywords: Diazepam, Flumazenil, Gastrointestinal motility, Morphine, Naloxone, Withdrawal symptoms

Various drugs in clinical practice when administered chronically can produce tolerance and dependence. There have been numerous reports of withdrawal syndrome manifestations upon abrupt cessation of these drugs in patients. Benzodiazepines are most prescribed drugs in clinical medicine today. They are given in the therapeutic management of anxiety, insomnia, muscle rigidity and convulsions. Benzodiazepines like other sedative hypnotics, induced tolerance and dependence¹, more especially as treatment with benzodiazepine is extended for longer periods. Abrupt cessation leads to excessive anxiety, tremor, muscle cramps, appetite, weight loss, gastrointestinal distress (diarrhoea) and convulsions^{2,3}. The discovery of specific high affinity BZ receptors has triggered considerable research relating to the central action of these compounds. Indeed, BZ binding sites have been identified not only in the CNS^{4,5}, but also in several peripheral tissues⁶.

Similarly, withdrawal states in morphine-dependent animals produce characteristic physiological and behavioral changes. This appearance of typical narcotic withdrawal signs can be elicited by cessation of morphine administration or precipitated by a treatment with a narcotic antagonist such as naloxone. Besides other numerous behavioral features such as jumping, wet dog shakes and teeth chattering, diarrhea is a major abstinence sign often used to quantify withdrawal response, which is opposite to the acute effects of morphine in naive animals. Mainly opioid inhibits secretion of fluid and electrolytes and stimulate gut smooth muscle secretions. Both effects are produced by interaction with different types of opioid receptors in the gut and the central nervous system. However, there is considerable controversy regarding the extent and importance of anatomical site involved in the effects of opioid on the gastrointestinal tract as well as on the predominant type of opioid receptors at each anatomical site. It is of great interest to examine intestinal motility associated with precipitated withdrawal in rats and mice made tolerant to

morphine and subsequently withdrawn, with naloxone.

With this background, the present study has been conducted in animals made dependent to drugs like diazepam or morphine to determine the percent inhibition induced by precipitated withdrawal in drug dependent animals (acute and chronic) and also to correlate these changes with the alterations observed in the propulsion of luminal contents and lastly to determine whether central and or peripheral receptors are involved by using diverse antagonists to trigger the syndrome of abstinence.

Materials and Methods

Albino mice (Laka strain) of male sex weighing between 20-30 g bred in Central Animal House (CAH) facility of Panjab University, Chandigarh were used. Animals were acclimatized to laboratory conditions before the experiment. All the experiments were carried out between 0900 and 1700 hr, using a randomized design. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Experimental procedure

Diazepam treatment schedule and effect of Ro 15-1788 (flumazenil) on diazepam withdrawal in mice—Diazepam (20 mg/kg, ip) was administered twice daily for 7 days. Control animals receive proportional volume of saline. The body weights of animals were recorded every day. On completion of chronic treatment with diazepam for 7 days, the animals were divided into two different groups for further studies; each group consisted of minimum of six animals. On day 7, feed was withheld and on day 8 i.e. 24 hr after the last dose of diazepam, withdrawal symptoms were observed.

The diazepam-withdrawn animals were divided into two groups. One group received no drug on day 8 while second group received Ro 15-1788 (1 mg/kg, ip) on day 8 i.e. exactly 24 hrs after the last dose of diazepam.

In another set of experiment, diazepam (20 mg/kg, ip) was administered once and animals were observed for acute effect after half an hour of drug treatment.

Measurement of withdrawal symptoms—Anti-anxiety effect: The withdrawal symptoms were measured using elevated plus-maze for measuring

anti-anxiety effect. The elevated plus-maze, developed by Pellow and File⁷ and modified by Kulkarni and Sharma⁸, is a novel test and for selective identification of anxiogenic and anxiolytic drug effects in rodents. The elevated plus maze consisted of two open arms (16 × 5 × 12 cm) and two closed arms (16 × 5 × 12 cm) with an open roof. The maze was elevated to a height of 25 cm from the floor. The animal was placed individually at the end of either of the open arms. During the 5 min test, the preference of the animal for the first entry, the number of entries into the open or closed arms and the time spent in each arm of the maze were recorded.

The readings were recorded on day 1 i.e. 30 min after diazepam treatment and on day 8 i.e. in chronically diazepam dependent mice challenged to Ro 15-1788 treatment.

Charcoal meal test: The charcoal meal test was used to assess the effect of benzodiazepine on intestinal transit⁹. Briefly, mice were starved for 24 hr prior to the experiment but had free access to water. To one group, no drug was given on 8th day and to other group Ro 15-1788 (1mg/kg, ip) was administered. To both the groups a charcoal meal (0.20 ml/mouse consisting of 10 % charcoal in 5 % gum acacia) was administered orally. The animals were sacrificed 15 min after charcoal administration. The abdomen was opened and the entire small intestine starting from the pyloric end was removed and placed on the blotting paper. All care was taken to prevent any damage to the tissue. The total length of the small intestine and the distance traveled by the charcoal was measured and expressed as percentage inhibition.

$$\text{Inhibition (\%)} = \frac{\text{total length of intestine (cm)} - \text{distance travelled by charcoal (cm)}}{\text{total length of intestine}} \times 100$$

Morphine treatment schedule and effect of naloxone—Morphine (10 mg/kg, sc) was administered twice daily for 9 days. Control animals receive proportional volume of saline. The body weights of animals were recorded every day. On completion of chronic treatment with morphine for 9 days, the animals were divided into two different groups for further studies; each group consisted of minimum of six animals. On the 9th day, 1hr after morphine administration, withdrawal symptoms were observed.

In another group of animals on 10th day i.e. 18 hr after the last dose, naloxone (2 mg/kg, ip) was administered and animals were observed for naloxone precipitated withdrawal symptoms.

Morphine (10 mg/kg, sc) was administered once and animals were observed for acute effect after an hour of drug treatment.

Measurement of withdrawal symptoms—Hot plate method: The nociceptive threshold was measured by hot plate method elicited in response to noxious thermal heat as described by Eddy and Leimbach¹⁰. Animals were individually placed on the hot plate maintained at constant temperature ($55^{\circ} \pm 1^{\circ}\text{C}$) and the reaction of animals such as paw licking or jump response was taken as the end point. Baseline latencies of paw licking or jump responses from hot plate were established observing a cut off time of 10 sec to prevent injury to the paw.

Readings were recorded on day 1, 3, 6, 9 of chronic administration of morphine and on day 10th in morphine dependent mice challenged by naloxone treatment.

Intestinal motility—Charcoal meal test was done in three groups of animals i.e. acute morphine administered mice; chronically morphine administered mice after 9 days and on the 10th day to the naloxone challenged mice.

Drugs—Morphine sulphate (Pharma Chemico Laboratories, Chandigarh), naloxone (Sigma, USA), diazepam (Sigma, USA), Ro 15-1788 or flumazenil (Sigma, USA). Diazepam was dissolved in one drop of Tween 80 and solution was made with sterile water. Ro 15-1788 was dissolved in dimethylsulfoxide (DMSO) with a few drops of Tween 80. Rest other drugs were dissolved in distilled water. All the drugs were administered intraperitoneally in a constant volume of 1 ml/100 g body weight except activated charcoal (0.2 ml), which is given orally or morphine which was administered subcutaneously. Each group comprised minimum of 6 animals.

Statistical analysis—Data were expressed as mean $\pm$ SE and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered as statistically significant.

Results:

Effect of chronic diazepam treatment on weight gain and other behavioral activities in mice—Animals developed tolerance to acute sedative

effect of diazepam on chronic administration of the drug (20 mg/kg/day) twice a day for 7 days. The sluggish locomotor activity seen on acute treatment disappeared as animals moved normally in the home cages on chronic treatment (personal observation). Chronic treatment resulted in weight gain due to hyperphagia (Table 1).

Effect of diazepam withdrawal symptoms in mice addicted with chronic diazepam treatment—On abrupt termination of daily injections of diazepam at the end of 7th day, the animals manifested hyperlocomotion and hyperreactivity considered as severe anxiety (Table 2). Besides increase in anxiety levels, increase in intestinal motility was observed in all the drug-withdrawn animals (Table 3). Animals were hyperirritable showing vocalization (squeaking) on touching.

Effect of Ro 15-1788 on diazepam withdrawal anxiety levels and intestinal motility—Ro 15-1788 treatment (1 mg/kg) significantly precipitated

Table 1—Effect of chronic treatment of diazepam on body weight of mice.

[Values are mean $\pm$ SE]

Days	Body wt (g)	Increase in body weight (%)
1	19.5 $\pm$ 0.80	-
2	19.8 $\pm$ 0.83	1.5
3	22.5 $\pm$ 0.62	15.3
4	23.8 $\pm$ 0.40	22.05
5	25.1 $\pm$ 0.30	28.7
6	27.3 $\pm$ 0.33	40
7	29.8 $\pm$ 0.55	52.8

Table 2—Effect of diazepam (acute, chronic and withdrawal precipitate, Ro 15-1788 treatment) on antianxiety effect measured as average time spent in closed arm. The activity was measured on elevated plus-maze for a 5 min

[Values are mean $\pm$ SE from 6 animals in each group]

Treatment (dose, mg/kg, ip)	Average time spend in closed arm (sec)
Control	84.3 $\pm$ 1.65
Acute diazepam treatment (20 mg/kg)	19.5 $\pm$ 1.73
Chronic diazepam treatment (20 mg/kg $\times$ 2 times $\times$ 7 days)	78.3 $\pm$ 1.67 [#]
Chronic diazepam treatment (20 mg/kg $\times$ 2 times $\times$ 7 days) + Ro 15-1788 (1 mg/kg) given on 8 th day**	193.8 $\pm$ 3.7 ^{*#}

**Please see details in text

P values: * < 0.001 compared with control, [#] < 0.001 compared with acute diazepam treatment

diazepam withdrawal. A reduction in both types of activities was recorded i.e., antianxiety levels and intestinal motility (Table 2, 3).

Effect of chronic morphine treatment on nociception in mice—Animals developed tolerance to acute antinociceptive effect of morphine on chronic administration of the drug (10 mg/kg/day) twice a day for 9 days. The peak antinociceptive effect that was seen on day 1 of morphine treatment disappeared on subsequent days (day 6 and 9) as measured by hot plate method (Fig. 1).

Effect of morphine withdrawal symptoms in mice addicted with chronic morphine treatment—On abrupt termination of daily injections of morphine at the end of 9th day, the animals manifested hyperalgesia on hot plate (Fig. 1). Besides hyperalgesia, increased gastrointestinal transit was observed on the very first day of morphine withdrawal (Table 4).

Effect of naloxone on morphine withdrawal symptoms in mice—Naloxone treatment (2 mg/kg) on 10th day i.e. 18 hr after the morphine withdrawal significantly precipitated the morphine withdrawal. Hyperalgesia as well as increased gastrointestinal transit was observed (Fig. 1, Table 4).

Table 3—Effect of diazepam (acute, chronic and withdrawal precipitate, Ro 15-1788 treatment) on gastrointestinal motility in mice (charcoal meal test) calculated as % inhibition [Values are mean $\pm$ SE from 6 animals in each group]

Treatment (dose, mg/kg, ip)	Acute gastric transit (cm)	Inhibition ^a (%)
Control	15.2 $\pm$ 0.35	70.33 $\pm$ 1.41
Acute diazepam treatment (20 mg/kg)	6.66 $\pm$ 0.76	87 $\pm$ 1.53
Chronic diazepam treatment (20 mg/kg $\times$ 2 times $\times$ 7 days)	19.6 $\pm$ 0.99	61.6 $\pm$ 1.20 [#]
Chronic diazepam treatment (20 mg/kg $\times$ 2 times $\times$ 7 days) + Ro 15-1788 (1 mg/kg) given on 8 th day**	28.5 $\pm$ 2.58	31.60 $\pm$ 2.34*•

^aFor details see text, **Please see details in text

P values: * $<$ 0.01 compared with control, ** $<$ 0.001 compared with acute diazepam treatment, # $<$ 0.05 compared with acute diazepam treatment

Discussion

Addiction and withdrawal—Physical dependence is a state that develops as a result of the adaptation (tolerance) produced by a resetting of homeostatic mechanism in response to repeated drug use. Drugs can affect numerous systems that previously are in equilibrium; these systems must find a new balance in presence of inhibition or stimulation by a specific drug. A person in this adapted or physically dependent state requires continued administration of the drug to maintain normal function. If administration of the drug is stopped abruptly, there is another imbalance and the affected system(s) must

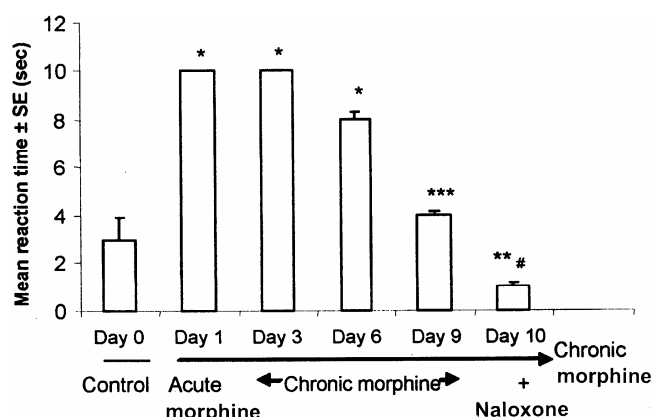


Fig. 1—Antinociceptive effect of morphine on day 1 (acute), day 3, 6 and 9 (chronic) and day 10 (withdrawal) after naloxone treatment. *P values: $<$ 0.001 as compared to control. ** $<$ 0.01 as compared with control. *** $<$ 0.01 as compared with morphine treatment (Day 1). # $<$ 0.05 as compared to control. **** $<$ 0.1 as compared with morphine treatment (Day 1).

Table 4—Effect of morphine (acute, chronic and withdrawal precipitate, naloxone treatment) on gastrointestinal motility in mice (charcoal meal test) calculated as % inhibition. [Values are mean $\pm$ SE from 6 animals in each group]

Treatment (dose, mg/kg, ip)	Acute gastric transit (cm)	Inhibition ^a (%)
Control	15.2 $\pm$ 0.35	70.33 $\pm$ 1.41
Acute morphine treatment (10 mg/kg)	9.16 $\pm$ 0.94	81.60 $\pm$ 1.43*
Chronic morphine treatment (10 mg/kg $\times$ 2 times $\times$ 9 days)	19.3 $\pm$ 1.84	61.00 $\pm$ 2.82*•
Chronic morphine treatment (10 mg/kg $\times$ 2 times $\times$ 9 days) + naloxone (ip) given on 10 th day**	25.5 $\pm$ 0.84	50.60 $\pm$ 0.88*•

^aFor Details See text, **Please see details in text

P values: * $<$ 0.001 compared with control, • $<$ 0.001 compared with acute morphine treatment, # $<$ 0.01 compared with control

again go through a process of readjusting to the new equilibrium without the drug. Many new drugs are coming into the market to prevent the drugs-induced withdrawal symptoms.

The appearance of a withdrawal syndrome when administration of the drug is terminated is the only actual evidence of physical dependence. Withdrawal signs and symptoms occur when drug administration in a physically dependent person is abruptly terminated. Withdrawal symptoms are characteristic for a given category of drugs. Recent studies have shown the protective effect of bupropion (Atypical antidepressant) on morphine tolerance and dependence in mice¹¹.

The present results highlighted that the long-term treatment with addictive drugs (diazepam or morphine) as well as precipitated withdrawal symptoms by administering their respective antagonists, which are able to induce gastrointestinal changes.

Ro 15-1788 (Flumazenil) on diazepam withdrawal in mice—Benzodiazepines, particularly, diazepam are one of the most widely prescribed drugs. They were at one time considered to be the safest CNS depressant drugs without abuse liability but subsequent studies have shown that they are capable of producing physical dependence with characteristic withdrawal syndrome in experimental animals and in man^{12,13}. Withdrawal reactions tend to be severe and occur earlier in patients taking benzodiazepines of short half-life, such as triazolam and lorazepam. The withdrawal symptoms of benzodiazepines (BZs) are increased anxiety, irritability, sleep disturbances, increased muscle tone and sexual function, multifocal myoclonus and convulsions. With the availability of BZ antagonists it has been possible to precipitate withdrawal symptoms. In the present study, increased anxiety status when tested on elevated plus maze and increased gastrointestinal motility were observed on abrupt termination of chronic diazepam treatment. Withdrawal hyperanxiety and increase in gastrointestinal motility were precipitated by R0 15-1788 treatment in chronic BZ treated animals. The increase in anxiety levels is well explained but the increase in the withdrawal gastrointestinal motor effects can be explained by differences in target effect. The BZs interact with two distinct receptors namely the central benzodiazepine receptor (CBR) and the peripheral type benzodiazepine receptor (PBR). The CBR are the component of the GABA_A

receptor coupled chloride ionophore complex¹⁴ and through this receptor are expressed the anxiolytic and anticonvulsant properties of BZs whereas PBR are found on the membrane of several mammalian tissues including guinea pig ileal muscle as well as brain non-neuronal cells¹⁵. The ligands of PBR interact with voltage-gated Ca²⁺ channels. Diazepam binds with relatively high affinity to both CBR and PBR. Our results showed that Ro 15-1788 acted on central receptor in brain as well as PBR located in brain and in digestive tract to induce its motor intestinal effects.

Benzodiazepine allosterically modulated GABA_A receptor mediated chloride (Cl⁻) influx and the GABAergic sensitivity was altered after prolonged exposure to BZs^{16,17}. Besides, the effect of GABA on the gastrointestinal motility has also been documented. It has been shown that, in conscious rats, GABA_A receptor stimulation induced an inhibition of the duodenal-jejunal motility, whereas GABA receptor stimulation increased and disrupted the cyclic motility pattern¹⁸.

Naloxone on morphine withdrawal in mice—The constipating and antidiarrheal effects of exogenously administered opioids have been well documented. Opioids are known to inhibit secretion of fluid and electrolytes and increase the tone of gut smooth muscle. Withdrawal states in morphine dependent animals produced characteristic physiological and behavioral changes. A narcotic antagonist like naloxone can precipitate the typical narcotic withdrawal signs. Besides the numerous behavioral features, such as jumping, wet dog shakes and teeth chattering, diarrhoea is also a major abstinence sign often seen as withdrawal response and used to detect withdrawal to opiates. The aim of our study was to determine that if the antitransit effects of opioid during chronic administration were decreased when compared to those observed during acute administration. Preliminary experiments demonstrated that continuous exposure to morphine leads to rapid development of tolerance or decreased sensitivity, indicated by decrease in antinociceptive activity when tested over a period of 9 days. On abrupt cessation of morphine treatment and naloxone challenge (2 mg/kg, ip) resulted not only withdrawal jumps but also significant increase in intestinal motility on the 10th day. Naloxone *per se* was unable to affect the intestinal motility. The results are in agreement with the observations of Wharhurst *et al.*¹⁹ who have shown that opiates are well known to reduce *in vitro*

intestinal secretion by a direct action on intestinal opioid receptors^{20,21}; consequently it is tempting to suggest that peripheral administration of naloxone in morphine-treated rats may abruptly increase intestinal secretion. But it has been shown that sites within CNS are involved in the antisecretory and gastrointestinal activity of opioid peptides. Absence of any withdrawal syndrome and motor gut disturbances after ICI 154129 administration, a supposed to be a specific *delta* receptor antagonist²², reinforces the fact that predominantly *mu* receptor are involved in precipitated morphine withdrawal by naloxone²³. The decreased response to opioids after tolerance and dependence is suggested to be due to down regulation or desensitization of *mu* receptors. Significantly opioids produce their antitransit effects by interacting with both peripheral as well as central (spinal and supraspinal) receptors. The *mu* receptors located in gut and/or the spinal cord could mediate the decreased anti-transit effects of opioids during diarrhoea associated with opioid dependence and withdrawal.

Experimental and clinical findings have suggested the involvement of other receptors systems in opioid withdrawal. Adenoceptors mediated mechanisms are reported to be involved in the acute abstinence syndrome that follows abrupt opiate withdrawal^{24,25}. Further, previous studies on isolated ileum from tolerant guinea pig showed that withdrawal liberates a variety of neurotransmitters in the gut wall including acetylcholine, 5-HT and vasoactive intestinal polypeptides²⁶ which may be responsible for the contraction of intestinal muscle or for the intestinal hypersecretion. This result permit us to gain insight into the symptoms of gastrointestinal distress described in humans after abrupt cessation of treatment and explains signs of diarrhea seen in animals after precipitated withdrawal. The results of the study also suggest the involvement of both central and peripheral receptors of benzodiazepines, *mu* receptors in the withdrawal symptoms of the benzodiazepines or morphine respectively.

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References

- 1 Kalant H, Leblanc A E & Gibbons R J, Tolerance to and dependence on some non-opiate psychotropic drugs, *Pharmacol Rev*, 23 (1971) 135.
- 2 Pevnick J S, Jasinski D R & Haertzen CA, Abrupt withdrawal from therapeutically administered diazepam, Report of a case, *Arch Gen Psychiatry*, 35 (1978) 995.
- 3 Winokur A, Rickeles K, Greenblat D J & Synder P J, Withdrawal reaction from long-term low-dosage administration of diazepam, *Arch Gen Psychiatry*, 37 (1980) 101.
- 4 Mohler H & Okada T, Benzodiazepine receptors: Demonstration in the central nervous system, *Science*, 198 (1977) 849.
- 5 Squires R F & Braestrup C, Benzodiazepine receptors in rat brain, *Nature (Lond.)*, 266 (1977) 732.
- 6 Braestrup C & Squires R F, Specific benzodiazepine receptors in rat brain characterized by high affinity 3H-diazepam binding, *Proc Natl Acad Sci USA*, 74 (1977) 3505.
- 7 Pellow S & File S E, Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat, *Pharmacol Biochem Behav*, 24 (1986) 525.
- 8 Kulkarni S K & Sharma A C, Elevated plus-maze: a novel psychobehavioral tool to measure anxiety in rodents, *Methods Find Exp Clin Pharmacol*, 13 (1991) 573.
- 9 Bansinath M & Bhargava H N, Evidence for a peripheral action of thyrotropin releasing hormone on gastrointestinal transit in mice, *Neuropharmacology*, 27 (1988) 433.
- 10 Eddy N B & Leimbach D, Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines, *J Pharmacol Exp Ther*, 107 (1953) 385.
- 11 Joshi D, Singh A, Naidu P S, Kulkarni S K, Protective effect of bupropion on morphine tolerance and dependence in mice, *Methods Find Exp Clin Pharmacol*, 26 (2004) 623.
- 12 Petursson H & Lader M H, Withdrawal from long-term benzodiazepine treatment, *Br Med J (Clin Res Ed)*, 283 (1981) 643.
- 13 Murphy S M, Owen R T & Tyrer P J, Withdrawal symptoms after six weeks treatment with diazepam, *Lancet*, 2 (1984) 1389.
- 14 Barnard E A, Bilbe G, Houamed K, Moss S J, Van Renterghem C & Smart T G, Functional expression in the *Xenopus* oocyte of messenger ribonucleic acids encoding brain neurotransmitter receptors: further characterisation of the implanted GABA receptor, *Neuropharmacology*, 26 (1987) 837.
- 15 Marangos P J, Patel J, Boulenger J P & Clark Rosenberg R, Characterization of peripheral type benzodiazepine binding sites using ³H-Ro 5-4864, *Mol Pharmacol*, 22 (1982) 26.
- 16 Gallager D W, Lakoski J M, Gonsalves S F & Rauch S L, Chronic benzodiazepine treatment decreases post-synaptic GABA sensitivity, *Nature (Lond.)*, 308 (1984) 74.
- 17 Gallager D W, Rauch S I & Malcolm A B, Alterations in a low affinity GABA recognition site following chronic benzodiazepine treatment, *Eur J Pharmacol*, 98 (1984) 159.
- 18 Fargeas M J, Fioramonti J & Bueno L, Central and peripheral action of GABA_A and GABA_B agonists on small intestine motility in rats, *Eur J Pharmacol*, 15 (1988) 163.
- 19 Warhurst G, Smith G S, Higgs N, Tonge A & Turnberg L A, Influence of morphine tolerance and withdrawal on intestinal

- salt and water transport in the rat in vivo and in vitro, *Gastroenterology*, 87 (1984) 1035.
- 20 Kachur J F, Miller R J & Field M, Control of guinea pig intestinal electrolyte secretion by a delta-opiate receptor, *Proc Natl Acad Sci USA*, 77 (1980) 2753.
- 21 Mc Kay J S, Lanacker B D & Turnberg L A, Influence of opiates on ion transport across rabbit ileal mucosa, *Gastroenterology*, 80 (1981) 279.
- 22 Shaw J S, Miller L, Turnbull H J, Gormley J J & Morley J S, Selective antagonists at the opiate delta receptors, *Life Sci*, 31 (1982) 1259.
- 23 DeLander G E, Portoghese P S & Takemori A E, Role of spinal mu opioid receptors in the development of morphine tolerance and dependence, *J Pharmacol Exp Ther*, 231 (1984) 91.
- 24 Aghajanian G K, Tolerance of locus coeruleus neurons to morphine and suppression of withdrawal response by clonidine, *Nature (Lond.)*, 276 (1978) 186.
- 25 Gold M S, Radmond D E & Kleber M D, Clonidine blocks acute opiate withdrawal symptoms, *Lancet*, 2 (1978) 599.
- 26 Schultz R & Herg A, Aspects of opiate dependence in the myenteric plexus of the guinea pig, *Life Sci*, 19 (1976) 1117.