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The Behavioural Effects of Long-Term Use of Benzodiazepine Sedative and Hypnotic Drugs: What can be learned from Animal Studies?

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In 1992, the National Advisory Committee on Core Health and Disability Support Services published a report on the use of minor tranquilizers which advised that continued use of anxiolytic or hypnotic agents for 10 days or longer may result in dependence. The purpose of this review is to summarise and critically evaluate the evidence from animal studies relating to the development of tolerance to, and dependence upon, benzodiazepine (BDZ) anxiolytic and hypnotic drugs, and to determine whether this evidence is consistent with the current government warning. It is argued that there is substantial evidence to support the view that tolerance develops to the muscle relaxant, ataxic, locomotor and anticonvulsant effects of BDZs within 2–3 weeks of continuous administration; however, the evidence supporting the development of tolerance to their anxiolytic effects is less conclusive. Although there are few quantitative studies relating to dependence, the available evidence suggests that some BDZs may induce dependence even at low doses when treatment is maintained for 1–2 weeks. It is concluded that the evidence from animal studies is consistent with the current New Zealand government warning regarding the dependence liability of BDZ anxiolytic and hypnotic drugs.

Benzodiazepine (BDZ) compounds are used for many purposes: as anxiolytics, hypnotics, anticonvulsants, pre-anaesthetic sedatives, muscle relaxants and to reduce the symptoms of alcohol withdrawal (see Rall, 1991; Laverty, 1992; Woods, Katz & Winger, 1992; Shader & Greenblatt, 1993 for reviews). However, it is their widespread and, in some cases, excessive use as anxiolytic and hypnotic agents for which they are best known (Laverty, 1992; Shader & Greenblatt, 1993; see Table 1). Over the last decade, concern has increased regarding the extensive use and potential dependence liability of BDZs (Laverty, 1992; Woods, Katz & Winger, 1992; North, McAvoy & Powell, 1992; Shader & Greenblatt, 1993). Between 1981 and 1989, the world sales of BDZ tranquilizers and hypnotics increased by 14% and 47%, respectively (based on 31 selected countries; Woods, Katz & Winger, 1992). In 1989, BDZ compounds were estimated to command an 83% share of the world market in minor tranquilizer sales and a 41% share of the world market in sedative/hypnotic sales (Woods, Katz & Winger, 1992). In a study of an Auckland general practice, North, McAvoy and Powell (1992) estimated that overuse of BDZs by patients occurred with approximately 9% of BDZ prescriptions but that the frequency of BDZ prescriptions had probably declined since the mid-1980s. In 1992, in response to increasing concern about minor tranquilizer abuse, the New Zealand Government published a report advising that continued use of anxiolytic or hypnotic agents for as little as 10 days may result in dependence in approximately 45% of patients (The National Advisory Committee on Core Health and Disability Support Services, 1992).

Despite the widespread use of BDZs, relatively little is understood of their long-term effects on the central nervous system (CNS) and behaviour (see File, 1985; Lader & Petursson, 1983; Woods,

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Table 1: *Some Commonly Prescribed Benzodiazepines*

Drug	Anxiolytic	Hypnotic	Alcohol Withdrawal	Anti-convulsant	Pre-anesth.	Half-life (hs)	Dose (mg)
chlordiazepoxide (NOVA-PAM)	•	•		•		8-24	15-100
diazepam (VALIUM)	•		•	•	•	20-90	5-20
triazolam (HALCION)		•				2-5	.125-.25
temazepam (SOMAPAM)		•				10-20	10-30
oxazepam (SERAPAX)	•		•			10-25	10-60
lorazepam (ATIVAN)	•	•	•	•	•	10-20	3-10

Table Legend

Table I: Some benzodiazepine anxiolytic and hypnotic drugs which are commonly prescribed in New Zealand. New Zealand trade names are shown in brackets. According to North, McAvoy and Powell (1992), diazepam and lorazepam are the most frequently prescribed BDZ anxiolytic drugs in New Zealand and triazolam and temazepam are the most frequently prescribed hypnotics. The doses listed are the usual, daily dose ranges for adults (from New Ethical Catalogue, 1993); in the case of anxiolytic therapy the total dose would normally be given in divided doses. Anti- convul., anticonvulsant; pre- anesth., pre-anesthetic; alcohol withdr., alcohol withdrawal; hs, hours. Modified from Rall (1991), North, McAvoy and Powell (1992) and Shader and Greenblatt (1993).

Katz & Winger, 1992 for reviews). It is clear that some humans do become dependent upon BDZs with repeated use (e.g. Winokur, Rickels, Greenblatt, Snyder & Schatz, 1980; Pevnick, Jasinski & Haertzen, 1988; Schweizer, Rickels, Case & Greenblatt, 1991; see Taylor, 1989 for a review); however, the conditions (e.g. dose and duration of treatment) which predispose certain individuals to dependence are yet to be elucidated (File, 1985; Taylor, 1989; Laverty, 1992; Woods, Katz & Winger, 1992; Shader & Greenblatt, 1993).

The advantage of drug testing in animals is presumed to be the facility to conduct strictly controlled investigations of the behavioural, neurophysiological and neurochemical effects of a particular drug, which may be difficult or impossible to do using human populations. In the case of BDZs there is a large literature on chronic effects in animals. However, due to differences in species, dose regimens, routes of administration and measurement methods used in the various studies, it is often difficult to draw general conclusions from the available literature and to understand how these conclusions may relate to BDZ therapy in humans. Many of the animal studies of BDZ tolerance and dependence are undermined by the use of crude rating scales to measure behavioural variables. Others have employed high doses of BDZs which, even allowing for differences in metabolism between rodents and humans, bear no relationship to those used in clinical situations. Relatively few animal studies have directly addressed BDZ dependence because of the difficulty in accurately measuring the withdrawal syndrome.

The aim of the present review is to critically evaluate the evidence from animal studies relating to the development of BDZ tolerance and dependence, and to determine what the animal literature can contribute to the understanding of BDZ use and abuse in humans. Although several recent reviews of the effects of BDZs in humans are available (e.g. Woods, Katz & Winger, 1992; Laverty, 1992; Shader & Greenblatt, 1993), few reviews of the current animal literature are available (e.g. Lader & Petursson, 1983; File, 1985).

Definitions of Drug Tolerance and Dependence

'Drug tolerance' is defined as the process in which the effects of a given dose of a drug decrease with repeated administration, resulting in the need to use higher doses in order to achieve the same behavioural effect (Rall, 1991). Drug tolerance may be attributable to metabolic changes ('dispositional or pharmacokinetic tolerance'; Rall, 1991) or plasticity in the CNS, reflecting adaptive neural changes which have occurred as a result of drug experience ('pharmacodynamic

tolerance'; Rall 1991). Sometimes, the observed drug tolerance is partly pharmacokinetic and partly pharmacodynamic. In the case of BDZs, there is substantial evidence from animal studies to indicate that a major component of the observed tolerance is pharmacodynamic in origin: in most cases, the development of tolerance does not correlate with a decrease in blood plasma, cerebrospinal fluid (CSF) or brain levels of BDZs as would be expected if tolerance were due to increased metabolism (see Table 2). Therefore a large component of BDZ tolerance is due to plasticity within the CNS.

'Drug dependence' is usually defined as a condition in which a withdrawal syndrome is induced when drug administration terminates (e.g. Rall, 1991). Although traditionally a distinction has been made between 'physiological' and 'psychological' dependence, this distinction has become increasingly difficult to sustain as the physiological relationship between the various withdrawal symptoms becomes recognised. Nonetheless, the more cognitive and emotional aspects of withdrawal syndromes are notoriously difficult to define and measure and for this reason fewer quantitative studies of these aspects of dependence have been conducted using animals (Busto & Sellers, 1991).

Although, in general, tolerance is not necessarily causally related to dependence, in the case of BDZs there is considerable evidence to support the hypothesis that the CNS adaptations which develop during long-term BDZ administration are related to the withdrawal syndrome which occurs following abstinence (e.g. Miller, Greenblatt, Beth Roy, Summer & Shader, 1988). However, the development of tolerance is a stratified process in which tolerance to the different behavioural effects of BDZs develops at different rates and to different extents.

Consequently, the withdrawal symptoms which are elicited following cessation of BDZ treatment may depend on the particular forms of tolerance which have developed up to the time that the drug administration is discontinued.

Comparisons Across Species: What are the Limitations?

There are several important limitations on the extent to which results from animal studies can be applied to human BDZ therapy. Most of the animal research has been conducted using species which metabolise BDZs very rapidly compared to humans. Because of these metabolic differences, in animal studies it is difficult to administer doses of BDZs which are similar to those used in humans. On a mg/kg basis the doses administered to humans seem low compared to those used in animal studies (e.g. 20 mg diazepam for a 70 kg human adult equals 0.29 mg/kg [Shader & Greenblatt, 1993] compared to doses of 5 mg/kg or greater often used in animal studies [Davis & Gallagher, 1988]). However, the elimination half-life of many BDZs is at least 10 times longer in humans than in rodents (Friedman, Abernathy, Greenblatt & Shader, 1986). In order to maintain blood plasma levels of BDZs which are similar to those which occur in humans undergoing BDZ therapy, some researchers have used methods which permit continuous administration of the BDZ (e.g. subcutaneous (s.c) silastic capsules, Davis & Gallagher, 1988). However, the use of continuous infusion or single daily injections results in a very different pattern of drug administration to that used in humans, where BDZs are often taken in divided daily doses (e.g. 20 mg divided into 2 daily doses, Shader & Greenblatt, 1993).

The route of BDZ administration is another variable which often differs greatly between animal studies and human clinical therapy: in most cases humans take BDZs orally and therefore active metabolites produced by first-pass metabolism in the liver may contribute to the behavioural effects of the drug (Rall, 1991). By contrast, many animal studies have used routes of administration which are not subject to first-pass metabolism (e.g. s.c injection or continuous infusion by silastic capsule).

Does this mean that it is impossible to use animals to simulate the BDZ therapy used in humans and that therefore animal studies are irrelevant to understanding the effects of such therapy? No, it means that there are limitations on the extent to which generalizations can be made from the animal studies to human BDZ therapy. However, more realistic animal models of BDZ treatment in humans can be developed by recognising these limitations and minimising differences (for e.g., by using blood plasma measurements to achieve BDZ levels similar to those used in humans). While behavioural studies of the effects of BDZs in rodents cannot replace similar studies in humans, a detailed understanding of the neuronal effects of chronic BDZ treatment can only be achieved through the use of animal models of human BDZ therapy. Only in this way is it possi-

Table 2: *Pharmacokinetic Correlates Of Tolerance*

Study	Species	BDZ Treatment (mg/kg)	Pharmacokinetic Correlate of Tolerance
Muscle relaxant and ataxic effects			
Tyma et al (1984)	cat	flurazepam (5) gastric tube daily for 36d	increased BDZ-like activity in cerebrospinal fluid
Rosenberg and Chiu (1982)	cat	flurazepam (2-20) gastric tube daily for 35d	half-life of the flurazepam metabolite, norflurazepam, increased
Locomotor effects			
File (1981)	rat	lorazepam (.25, .5) triazolam (.075, .25) i.p daily for 3d	blood plasma levels reduced by 36% (lorazepam) and 31% (triazolam)
File (1982a)	rat	chlordiazepoxide (5-50) i.p daily for 5d	no change in blood plasma levels of chlordiazepoxide
File (1982b)	rat	lorazepam (.25, .5) i.p daily for 3d	no change in blood plasma levels of lorazepam for low dose, but decrease for high dose
Lister et al. (1983)	rat	lorazepam (.125-.5) i.p daily for 3d or (.5) i.p once every 2d	no change in blood plasma or brain levels of lorazepam
Anticonvulsant effects			
Frey et al. (1984)	dog	diazepam (.25, .5) orally, 3 times daily for up to 22d chlorazepate (1) orally, 3 times daily for 2w	no reduction in blood plasma levels of diazepam or chlorazepate
Scherkl et al. (1985)	dog	clonazepam (.5) orally, daily for 3-6w	no reduction in blood plasma levels of clonazepam
Loscher and Schwark (1985)	rat	diazepam (5) i.p, 3 times daily for 2w	no reduction in blood plasma levels of diazepam
Haigh et al. (1986)	mouse	clonazepam (.08-.5) i.p twice daily for 16d	no reduction in blood plasma levels of clonazepam
Gallager et al. (1985)	rat	diazepam (approx. 5), per day, released continuously by s.c silastic capsule for 3w	no reduction in brain levels of diazepam
Gonsalves and Gallager (1988)	rat	diazepam (approx. 4 mg/day, irrespective of weight, released continuously by s.c silastic capsule)	no reduction in brain levels of diazepam
Anxiolytic effects			
Davis and Gallager (1988)	rat	diazepam (5) i.p, daily for 5d (5) i.p, daily for 21d (20) i.p, daily for 4d (5) per day, continuously released by s.c silicone capsule for 5d	no reduction in brain levels of diazepam

Table Legend

Table II: In this and the following Tables, d = days, w = weeks, i.p = intraperitoneal, s.c = subcutaneous, i.v = intravenous. Doses are given in brackets.

ble to understand the CNS mechanisms of pharmacodynamic tolerance and dependence and develop drug treatments which may be used to reverse the dependence process (e.g. Schweizer, Rickels, Case & Greenblatt, 1991; Prather, Rezazadeh, Lane, Rowan, Hooper & Lytle, 1993).

Evidence for Tolerance to Benzodiazepines

Muscle relaxant, ataxic and locomotor effects

The effects of BDZs on muscle relaxation, ataxia and locomotor activity are difficult to separate in many studies, since muscle relaxation usually causes ataxia, which in turn reduces locomotor activity. Therefore, this section will consider studies which have measured muscle relaxation and ataxia directly as well as those which have drawn inferences from open-field activity where other factors (e.g. anxiety, motivation) may be involved.

There have been very few quantitative studies of the chronic effects of BDZs on muscle relaxation and ataxia. In most cases muscle relaxation has been assessed qualitatively using rating scales, which may underestimate the variability between animals (see Table 3). In general, tolerance to the muscle relaxant and ataxic effects of BDZs has been reported to develop rapidly, within 2 weeks of chronic BDZ treatment (see Table 3); in the case of divided doses, tolerance may develop within a few days (Smith & Darlington, 1994; see Table 3). Diazepam, flurazepam and chlordiazepoxide have all been tested and there seem to be no obvious differences in the capacities of these compounds to induce tolerance to their muscle relaxant and ataxic effects. However, it should be noted that most of the studies relating to the effects of flurazepam have used very high doses (e.g. 100–150 mg/kg/day, see Table 3) and it is questionable whether the results of these studies

Table 3: *Tolerance to Muscle Relaxant and Ataxic Effects*

Study	Species	BDZ Treatment (mg/kg)	Tolerance Observed
Hoogland et al. (1966)	rat	chlordiazepoxide (100) i.p, daily for 5d	approx. 5d (muscle relaxation; handling)
Goldberg et al. (1967)	rat	chlordiazepoxide (150) oral, (50) i.p daily for 2w	approx. 2w (ataxia; rotarod)
	mouse	(100) oral, (50) i.p daily for 2w	approx. 2w (ataxia; rotarod)
Rosenberg and Chiu (1981a)	rat	flurazepam (100-150) orally, daily for 1-8w	4w (ataxia; rating scale)
Rosenberg and Chiu (1982)	cat	flurazepam (2-20) gastric tube, daily for 35d	approx. 2-3d (muscle relaxation; ataxia for 5-20 mg/kg; rating scales)
Matsubara and Matsushita (1982)	rat	diazepam (2.5-20) orally, daily for 2w	2d (muscle relaxation; inclined screen, handling)
Rosenberg et al. 1983	rat	flurazepam (100-150) orally, daily for 4w	approx. 4w (ataxia; rating scale)
Mele et al. (1984)	rat	diazepam (10) i.v, daily for 10d	no tolerance (muscle relaxation, RR; handling)
Tyma et al. (1984)	cat	flurazepam (5) gastric tube, daily for 36d	approx. 2w (muscle relaxation; rating scale)
Tietz and Rosenberg (1988)	rat	flurazepam (100-150) daily, orally for up to 4w	1w (turning in response to intranigral injection of flurazepam)
Hutchinson et al. (1993)	guinea pig	diazepam (2) i.p, daily for 6w	approx. 2-3w for 2/4 animals (RR; load cell measurement)
Scott et al. (1994)	guinea pig	diazepam (5) i.p, daily for 3-4w	no tolerance (RR; measured electronically)
Smith and Darlington (1994)	guinea pig	diazepam (5) i.p, 3 times daily for 5d	3d (RR; load cell measurement)

Table Legends

Tables III, IV, V, VI: Abbreviations as for Table II. 'Tolerance observed' indicates the approximate time until tolerance was observed, although in some cases tolerance was incomplete. The specific behavioural variable measured and the method used is given in brackets in the "tolerance observed' column. RR = righting reflex.

Table 4: *Tolerance to General Locomotor Effects*

Study	Species	BDZ Treatment (mg/kg)	Tolerance Observed
Goldberg et al. (1967)	rat	chlordiazepoxide (150) oral, (50) i.p daily for 2w	approx. 2w (locomotor activity; activity measurement system)
	mouse	(100) oral, (50) i.p daily for 2w	as above
Sansone (1979)	mouse	chlordiazepoxide (5-30) i.p daily for 5d	5d (locomotor activity; activity measurement system)
File (1981)	rat	lorazepam (.25, .5) triazolam (.075-.25) i.p daily for 3d	3d (locomotor activity, head dipping; holeboard apparatus)
Matsubara and Matsushita (1982)	rat	diazepam (2.5-20) orally, daily for 2w	2d (locomotor activity; open field test, activity measurement system)
File (1982a)	rat	chlordiazepoxide (5-50) i.p, daily for 5d	5d (locomotor activity, specific movements; holeboard apparatus)
File (1982b)	rat	lorazepam (.25) i.p daily for 3d	3d (locomotor activity, head dipping; holeboard apparatus)
Lister et al. (1983)	rat	lorazepam (.125-.5) i.p daily for 3d or (.5) i.p once every 2d	3d (locomotor activity, head dipping; holeboard apparatus)
Tyma et al. (1984)	cat	flurazepam (5) gastric tube, daily for 36d	approx. 2w (locomotor function; rating scale)
Harro et al. (1990)	rat	diazepam (5) i.p, daily for 2w	2w (open field test)

generalise to lower doses. One exception to the general trend in the above findings is the study by Mele, Sagratella and Massotti (1984), in which it was reported that tolerance to the muscle relaxant effects of diazepam in rats did not develop even after 10 days of intravenous (i.v) administration (10 mg/kg/day).

In general, studies of the effects of chronic BDZ administration on locomotor activity have demonstrated that tolerance to the initial depressive effects of BDZs develops rapidly (within 2 weeks, in some cases within 2–3 days; see Table 4). Similar results have been obtained with a number of BDZs, including chlordiazepoxide, lorazepam, triazolam, diazepam and flurazepam.

Anticonvulsant effects

Tolerance to the anticonvulsant effects of BDZs has been consistently reported, using either chemical (e.g. pentylenetetrazol) or electrical stimuli (ie. kindling) to induce seizures. However, the time frame for the development of this form of tolerance varies widely between different studies, depending on the seizure stimulus and BDZ dose regime used (see Table 5). In general, significant tolerance usually develops within 3 weeks. BDZs which have been investigated in this context include clobazam, diazepam, chlorazepate, clonazepam and chlordiazepoxide.

Anxiolytic effects

Experiments which have investigated the chronic effects of BDZs on anxiety have yielded a number of different results, depending on the BDZ and the measurement paradigm used (see Table 6).

Tolerance to the anxiolytic effects of chlordiazepoxide within 2–4 weeks has been reported in 2 studies which have used anxiety measures not involving shock (the social interaction test, Vellucci & File, 1979; elevated plus maze performance, Ishihara, Hiramatsu, Kameyama & Nabeshima, 1993). The results from studies investigating the effects of diazepam are inconsistent. Several studies using measurement paradigms involving electric shock have reported tolerance

Table 5: *Tolerance to Anticonvulsant Effects*

Study	Species	BDZ Treatment (mg/kg)	Tolerance Observed
Gent and Haigh (1983)	mouse	clobazam (2.5, 5) i.p, twice daily for 10d	approx. 4-7d (pentylenetetrazole-induced seizures; minimal dose to elicit a seizure)
File (1983)	mouse	diazepam (4) i.p, daily for up to 45d	5-45d, depending on dose and convulsant (pentylene-tetrazole or picrotoxin-induced seizures; minimal dose to elicit a seizure)
Frey et al. (1984)	dog	diazepam (.25-.5) orally, 3 times daily for up to 22d	1w (pentetrazole-induced seizures; minimal dose to elicit seizure; electrophysiol recordings)
		chlorazepate (1) orally, 3 times daily for 2w	approx. 2w (as above)
Gallager et al. (1985)	rat	diazepam (approx. 5) per day, released continuously by s.c silastic capsule for 3w	3w (bicuculline-induced seizures; minimal dose to elicit seizure)
Gent et al. (1985)	mouse	clonazepam (.25) i.p, twice daily for 3d	3d (pentylenetetrazole-induced seizures; minimal dose to elicit a seizure)
		clobazam (10), as above	1d (as above)
Scherkl et al. (1985)	dog	clonazepam (.5) orally, daily for 3-6w	1-2w (pentetrazol-induced seizures; minimal dose to elicit a seizure)
Loscher and Schwark (1985)	rat	diazepam (5) i.p, 3 times daily for 2w	approx. 2w (seizures induced by amygdala stimulation after kindling; electrophysiol measurement)
Haigh et al. (1986)	mouse	clonazepam (.08-.5) i.p twice daily for 16d	4d (pentetrazol-induced seizures; minimal pentetrazol dose to elicit a seizure)
Gonsalves and Gallager (1988)	rat	diazepam (approx. 4 mg/day, irrespective of weight, released continuously by s.c silastic capsule)	approx. 1w (bicuculline-induced seizures; minimum dose to induce a seizure)
Garratt et al. (1988)	mouse	clordiazepoxide (12.5) or midazolam (.75) i.p twice daily for 15d; or nitrazepam (.6) i.p twice daily for 6d	15d (pentylenetetrazole-induced seizures; minimal dose to elicit seizure)
			6d (as above); tolerance incomplete in all cases
Mana et al. (1991)	rat	diazepam (2) i.p every 2d for 20d	22d (seizures induced by amygdala stimulation after kindling; manual measurement of forelimb clonus)

to the anxiolytic effects of diazepam (1–5 mg/kg/day) within 6–10 days (Stephens & Schneider, 1985; Treit, 1985). Other studies using similar diazepam dose regimens but not using shock to induce anxiety, have failed to obtain tolerance (e.g. social interaction, de Angelis & File, 1979; startle reflex amplitude, Davis & Gallagher, 1988; elevated plus maze, Harro, Lang & Vasar, 1990; see Table 6). However, Davis and Gallagher (1988) did obtain tolerance to the anxiolytic effects of diazepam on startle reflex amplitude when using high daily intraperitoneal (i.p) doses (20 mg/kg) or continuous release of diazepam (approx. 5 mg/24 hs) from s.c silastic capsules.

Table 6: *Tolerance to Anxiolytic Effects*

Study	Species	BDZ Treatment (mg/kg)	Tolerance Observed
Margules and Stein (1968)	rat	oxazepam (20) i.p, daily for up to 22d	approx. 7d (rate of unpunished behaviour; Skinner box)
de Angelis and File (1979)	mouse	diazepam (1) i.p, daily for 9d	no tolerance (social interaction in high levels of illumination; scored by [blind] observers).
Vellucci and File (1979)	rat	chlordiazepoxide (5) i.p, daily for 5-25d	approx. 25d (social interaction in high levels of illumination; scored)
Stephens and Schneider (1985)	mouse	diazepam (5) orally, daily for 9d	6d (rate of activity punished by footshock; 4 plate test)
Treit (1985)	rat	diazepam (1) i.p, daily for 10d	10d, only with high intensity shock (duration of defensive burying following shock)
Davis and Gallager (1988)	rat	diazepam (5) i.p, daily for 5d	no tolerance (startle reflex, measured electronically)
		(5) i.p, daily for 21d	no tolerance (as above)
		(20) i.p, daily for 4d	4d (method as above)
		(5) per day, continuously released by s.c silicone capsule for 5d	5d (method as above)
Harro et al. (1990)	rat	diazepam (5) i.p, daily for 2w	no tolerance (elevated plus maze)
Ishihara et al. (1993)	mouse	chlordiazepoxide (30) i.p, daily for 10-14d	14d (elevated plus maze)

These authors suggest that tolerance develops more easily and rapidly when CNS BDZ binding sites are occupied for long periods of time and that because of the short elimination half-life of diazepam in species such as rat (Friedman, Abernathy, Greenblatt & Shader, 1986; Klotz, Antonin & Bieck, 1976), single i.p injections are less likely to induce tolerance. Nonetheless, tolerance to the anxiolytic effects of chlordiazepoxide has been reported to occur within 4 weeks using doses as low as 5 mg/kg i.p in single daily injections (Vellucci & File, 1979); although chlordiazepoxide may penetrate the brain more slowly than diazepam (Van Der Kleijn, 1968), it has a shorter half-life (Rall, 1991). Therefore, the duration of the occupation of BDZ binding sites may not be equally important for the development of tolerance in the case of all BDZs.

In summary, the available evidence suggests that substantial tolerance to the muscle relaxant, ataxic, locomotor and anticonvulsant effects of BDZs develops within 2–3 weeks of continuous administration. By contrast, the data relating to the development of tolerance to the anxiolytic effects of BDZs are inconclusive.

Evidence for Dependence Upon Benzodiazepines

Relatively few studies have quantified the withdrawal syndrome following the cessation of BDZ treatment in animals. However, many of the withdrawal symptoms which have been observed (e.g. tremor, hyperactivity, listlessness, hyperthermia and reduced food intake) are similar to those which have been documented in humans during BDZ withdrawal (see Woods, Katz & Winger, 1992 for a review).

McNicolas, Martin and Cherian (1983) administered diazepam (60 mg/kg/day) or lorazepam (100 mg/kg/day) to dogs by gastric fistula for 2 weeks. Following abrupt termination of the drug treatment, a withdrawal syndrome occurred consisting of tremor, rigidity and reduced food intake. The withdrawal syndrome associated with diazepam was more intense than that associated with lorazepam, and included clonic and tonic-clonic seizures which proved lethal in some cases. Withdrawal syndromes following discontinuation of diazepam treatment have also been reported for rats and mice, using high doses (100–133 mg/kg/day by gastric fistula to rats for 2 weeks, Martin,

McNicholas & Cherian, 1982; 2.5–10.00 mg/kg i.p, twice daily, to mice for 7 days, Barry, Costall, Kelly & Naylor, 1987) to low doses (1mg/kg/day i.p to rats for 8 days, Eisenberg, 1987). The withdrawal syndromes observed in these studies consisted of jerks, tremors, wet dog shakes, reduced food and water intake, weight loss, increased hostility, explosive awakenings and increased locomotor activity (Martin, McNicholas & Cherian, 1982; Barry, Costall, Kelly & Naylor, 1987). Miller, Greenblatt, Beth Roy, Summer and Shader (1988) also observed withdrawal symptoms, measured as increased open field activity (i.e. distance travelled), following discontinuation of lorazepam treatment in mice. In this study the dose used (2 mg/kg/day for 7 days, by s.c osmotic minipump) was low compared to many studies and a direct relationship was observed between the development of tolerance and the onset of the withdrawal symptoms following discontinuation of the drug treatment. Other studies of lorazepam using the same dose regimen have reported that the withdrawal syndrome includes reduced seizure thresholds (Schatzki, Lopez, Greenblatt, Shader & Miller, 1989).

Studies by Scherkl and colleagues using dogs have shown that tolerance to low doses of clonazepam (0.5 mg/kg, orally, twice daily for 3–7 weeks) develops over 1–2 weeks and that following cessation of the drug treatment a withdrawal syndrome develops which consists of listlessness, wet dog shakes, tremor, dorsal recumbency, weight loss and hyperthermia (Scherkl, Scheuler & Frey, 1985; Scherkl & Frey, 1986). Similar low doses of alprazolam (2 mg/kg/day, s.c osmotic minipump for 7 days) have been shown to produce withdrawal syndromes in mice following discontinuation of the drug (Lopez, Miller, Greenblatt, Chesley, Schatzki & Shader, 1990; Galpern, Miller, Greenblatt, Szabo, Browne & Shader, 1991).

Ryan and Boisse (1983) administered chlordiazepoxide to rats intragastrically, twice daily for 35 days, adjusting the dose on a daily basis so that a high level of intoxication could be maintained (dose range 163.3–839.3 mg/kg/day). Tolerance developed within 10 days and discontinuation of the drug resulted in a severe withdrawal syndrome with included tremors, muscle hypertonus, piloerection, myoclonic jerks, increased startle response and weight loss. However, it should be noted that these doses of chlordiazepoxide are extremely high and therefore the results of the study may not be applicable to lower doses.

Overall, these results suggest that a certain degree of BDZ dependence may develop with as little as 1 week of continuous administration of some BDZs. Although some studies have used high doses which may not be applicable to BDZ therapy in humans, others have used doses which are quite low given the rapid metabolism of BDZs in lower mammals (e.g. 2 mg/kg/day of alprazolam for 7 days, Lopez, Miller, Greenblatt, Chesley, Schatzki & Shader, 1990).

Mechanisms of Benzodiazepine Tolerance and Dependence

The majority of studies suggest that the different forms of tolerance to BDZs cannot be explained entirely in terms of pharmacokinetic change (e.g. increased metabolism by the liver; see Table 2); therefore, a significant component of BDZ tolerance is believed to be due to neuronal plasticity within the CNS (i.e. 'pharmacodynamic tolerance'). Studies of the mechanisms of BDZ tolerance and dependence in animals can be divided into 2 main categories: 1) electrophysiological studies, in which CNS neurons which have been exposed to chronic BDZ treatment are recorded and their sensitivity to BDZs and inhibitory neurotransmitters tested; 2) binding and other biochemical studies in which the number, affinity or efficacy of neurotransmitter receptors is measured. Since BDZs are known to bind to a specific site on the 'A' subtype of receptor complex for the major inhibitory amino acid neurotransmitter, γ-aminobutyric acid (i.e. the $GABA_A$ receptor, Tallman, Thomas & Gallager, 1978), most of the electrophysiological and biochemical studies have focused on the $GABA_A$ receptor and its associated BDZ binding site.

Gallager, Lakoski, Gonsalves and Rauch (1984) reported that daily i.p injections of 5 mg/kg diazepam for at least 3 weeks resulted in a decrease in the sensitivity of dorsal raphe neurons to iontophoretically applied GABA. Their binding studies indicated a reduction in the ability of GABA to enhance BDZ binding in the cerebral cortex; however, there were no changes in the number or affinity of BDZ binding sites.

Since this original study, the finding that chronic diazepam administration results in a subsensitivity of dorsal raphe neurons to GABA has been replicated many times, both *in vivo* (Gonsalves & Gallager, 1987; Gallager, Malcolm, Anderson & Gonsalves, 1985; Gonsalves & Gallager, 1985; Gonsalves & Gallager, 1988) and *in vitro* (Wilson & Gallager, 1988; Hernandez, Heninger,

Wilson & Gallager, 1989). The development of GABAergic subsensitivity has been correlated with the development of tolerance to the anticonvulsant effects of diazepam (Gallager, Malcolm, Anderson & Gonsalves, 1985; Gonsalves & Gallager, 1987; Gonsalves & Gallager, 1988).

Although the development of a GABAergic subsensitivity in the dorsal raphe may provide an explanation for tolerance to the anticonvulsant effects of BDZs, not all areas of the CNS show this type of neuronal adaptation. Wilson and Gallager (1987; 1989) reported that chronic diazepam treatment did not reduce the sensitivity of substantia nigra pars reticulata neurons to GABA, although the ability of BDZs to enhance the effects of GABA was reduced. Tyma, Rosenberg, Tietz and Chiu (1988) also reported that chronic flurazepam treatment reduced the effects of flurazepam on substantia nigra pars reticulata neurons. A reduction in the facilitation of GABA-mediated inhibition by diazepam has been reported following chronic exposure of spinal cord cultures to diazepam (Sher, Study, Mazzetta, Barker & Nelson, 1983). Given the differences in the rate of development and extent of the various forms of BDZ tolerance, it is not surprising that different areas of the CNS undergo distinct changes in response to chronic BDZ treatment.

Recent studies suggest that the hippocampus, like the dorsal raphe, may develop a subsensitivity to GABA during the development of BDZ tolerance. Xie and Tietz (1992) reported that selective $GABA_A$ agonists (e.g. isoguvacine), but not the $GABA_B$ agonist baclofen, showed a 2-fold reduction in their ability to inhibit CA1-evoked field potentials in hippocampal slices removed from rats which had received chronic flurazepam treatment (an average of 100 mg/kg/day for 7 days, orally). Diazepam also had less effect on hippocampal field potentials in animals which had developed tolerance to flurazepam. A reduction in GABAergic inhibition was also suggested by a decrease in paired-plus inhibition in the CA1 region (Xie & Tietz, 1991).

If chronic BDZ treatment results in reductions in the sensitivity of some CNS neurons to BDZs and endogenous GABA, it might be hypothesised that these sensitivity changes would be reflected in changes in the number, affinity or efficacy of the $GABA_A$ and BDZ binding sites. Unfortunately, despite a large number of binding studies aimed at testing this hypothesis, the results are not clear. Some authors have reported a decrease in the number ('down-regulation') of BDZ binding sites following chronic administration of some BDZs (e.g. Chiu & Rosenberg, 1978; Rosenberg & Chiu, 1979; Rosenberg & Chiu, 1981; Crawley, Marangos, Stivers & Goodwin, 1982; Sher, Study, Mazzetta, Barker & Nelson, 1983; Szcawinska, Cenajek-Musial, Nowakowska & Chodera, 1988; Diana & Massotti, 1992; Byrnes, Miller, Greenblatt & Shader, 1993), others have reported no change in BDZ binding sites (e.g. Mohler, Okada & Enna, 1978; Braestrup, Nielsen & Squires, 1979; Gallager, Lakoski, Gonsalves & Rauch, 1984), an increase in the affinity of some $GABA_A$ binding sites for GABA (e.g. Gallager, Rauch & Malcolm, 1984; Gallager, Malcolm, Anderson & Gonsalves, 1985) or an increase in the number ('up-regulation') of $GABA_A$ binding sites (Ferrero, Guidotti & Costa, 1984). Some of these studies examined receptor binding in large areas of the forebrain and it is possible that in some cases the results were confounded by including different areas of the CNS which were exhibiting different changes. More recent studies which have compared different specific areas of the CNS have confirmed that, for some BDZs, a down-regulation of BDZ binding sites does occur in areas such as the cortex, hypothalamus and hippocampus (Miller, Greenblatt, Barnhill & Shader, 1988) but that the degree of down-regulation varies widely (Rosenberg & Chiu, 1981; Tietz, Rosenberg & Chiu, 1986). Many recent studies have suggested that BDZ tolerance and dependence are also associated with a reduction in the coupling of the BDZ and $GABA_A$ recognition sites, such that BDZs show reduced facilitation of GABA-mediated inhibition (e.g. Yu, Chiu & Rosenberg, 1988; Tietz, Chiu & Rosenberg, 1989; Allan, Baier & Zhang, 1992); this 'functional uncoupling' also appears to be regionally specific (Marley & Gallager, 1989) and may be the result of phosphorylation of the $GABA_A$ receptor by protein kinase C (Leidenheimer, Whiting & Harris, 1993). The discovery of a specific BDZ binding site on the $GABA_A$ receptor complex gave rise to speculation that there might be an endogenous BDZ in the CNS which modulates the sensitivity of the GABA binding site for GABA (e.g. Mocchetti & Santi, 1991); however, at present, the possible functional significance of an endogenous BDZ for the development of BDZ tolerance and dependence is unclear.

In summary, the majority of the evidence suggests that BDZ tolerance and dependence are at least partly due to plasticity in the CNS ('phar-

macodynamic tolerance'). The development of tolerance has been correlated with a subsensitivity to GABA in a number of areas of the CNS, most notably neurons in the dorsal raphe and the hippocampus; however, it is clear that some parts of the CNS (e.g. the substantia nigra) do not undergo such changes. Where a subsensitivity to GABA develops, it is not necessarily due to a down-regulation or reduced affinity of $GABA_A$ receptors; however, a reduction in the coupling of the $GABA_A$ and BDZ binding sites on the $GABA_A$ receptor complex seems likely.

At present it is not possible to resolve all of the discrepancies in the literature relating to receptor changes during the development of BDZ tolerance and dependence. However, if BDZ tolerance is associated with a reduction in GABAergic inhibition in some areas of the CNS (via the mechanisms described above), then it seems feasible that the withdrawal syndrome which follows the cessation of drug treatment may arise from the persistence of reduced GABAergic inhibition in the absence of the BDZ which induced this adaptation. However, the most severe phase of the BDZ withdrawal syndrome is correlated with an up-regulation of BDZ (Lopez, Miller, Greenblatt, Chesley, Schatzki & Shader, 1990; Miller, Greenblatt, Beth Roy, Summer & Shader, 1988) and GABA binding sites (Miller, Greenblatt, Beth Roy, Summer & Shader, 1988) and an increased Cl^- influx through the Cl^- channel associated with the $GABA_A$ receptor complex (Miller, Greenblatt, Beth Roy, Summer & Shader, 1988). Presumably, these receptor changes represent a compensatory response to the withdrawal symptoms generated by the reduced GABAergic inhibition and account for the gradual disappearance of the withdrawal syndrome (Miller, Greenblatt, Beth Roy, Summer & Shader, 1988). Interestingly, the development of BDZ tolerance and GABAergic subsensitivity can be prevented by a single injection of the selective BDZ antagonist Ro-151788 (flumazenil) during chronic BDZ administration (e.g. Gonsalves & Gallager, 1988) and once tolerance has developed, a withdrawal syndrome can be induced by an injection of flumazenil (e.g. Allan, Baier & Zhang, 1992). In some cases, a withdrawal syndrome can be prevented by flumazenil injections (Gallager, Heninger & Heninger, 1986; Baldwin & File, 1989). Recent studies suggest that the BDZ withdrawal syndrome can be substantially reduced by administration of the anticonvulsant carbamazepine (Galpern, Miller, Greenblatt, Szabo, Browne & Shader, 1991). Preliminary results suggest that carbamazepine may also be useful in reducing the BDZ withdrawal syndrome in humans (Schweizer, Rickels, Case & Greenblatt, 1991).

Conclusions

The major contribution which animal studies can make to more effective and safer BDZ therapy in humans is a better understanding of the mechanisms of BDZ tolerance and dependence. At present, there are limitations on the extent to which general conclusions can be drawn regarding the development of BDZ tolerance and dependence in experimental animals, given the many different BDZs, dose regimens, routes of administration, species, behavioural variables and measurement methods which have been used. Nonetheless, some conclusions of relevance to BDZ therapy in humans can be drawn.

Taken together, the available data from animal studies support the current New Zealand government warning that continuous BDZ administration for longer than 10 days carries the risk of dependency. There is convincing evidence that tolerance develops to the muscle relaxant, ataxic, locomotor and anticonvulsant effects of many BDZs over a period of 2–3 weeks of continuous administration (specific time course depending on the particular BDZ and the dose regimen used). However, the evidence supporting the development of tolerance to the anxiolytic effects of BDZs is more controversial and inconclusive (see Table 6). The available dependence studies suggest that dependence on some BDZs may develop with as little as 1 week of continuous administration.

Although tolerance may develop more readily with the continuous occupation of CNS BDZ binding sites afforded by divided doses or methods which allow continuous release of the BDZ (e.g. s.c silastic capsule, Davis & Gallager, 1988), tolerance can nonetheless develop with once daily injections of some BDZs, despite their short elimination half-life in lower mammals (Van Der Kleijn, 1969; Klotz, Antonin & Bieck, 1976; Klotz 1979; Friedman, Abernathy, Greenblatt & Shader, 1986). Some studies in humans suggest that tolerance may even develop with once weekly doses of BDZs such as lorazepam (e.g. File & Lister, 1983). BDZ tolerance cannot be attributed solely to pharmacokinetic factors: in most cases there is evidence for similar or even increased blood plasma or brain concentrations of the BDZ with continued administration, or direct evidence of pharmacodynamic changes in the CNS. A large

number of studies have shown that chronic BDZ administration results in a subsensitivity of neurons in some areas of the CNS to GABA and BDZs (e.g. Gallager, Lakoski, Gonsalves & Rauch, 1984); many biochemical studies suggest that chronic BDZ treatment may result in an area-specific down-regulation of BDZ binding sites (e.g. Miller, Greenblatt, Barnhill & Shader, 1988) and/or a reduced functional coupling between BDZ and GABA binding sites on the $GABA_A$ receptor complex (e.g. Yu, Chiu & Rosenberg, 1988). The withdrawal syndrome which is observed following discontinuation of BDZ treatment may be a result of reduced GABAergic inhibition in the absence of the BDZ which induced this change. These advances in the understanding of neuronal mechanisms responsible for dependence are contributing to the development of drug treatments which may prevent or reduce the BDZ withdrawal syndrome in humans (e.g. Schweizer, Rickels, Case & Greenblatt, 1991).

There is increasing evidence from animal studies that the $GABA_A$ receptor complex, including the BDZ recognition site, is modulated by environmental factors such as stress and handling (e.g. Biggio, Corda, Concas, Demontis, Rosetti & Gessa, 1981; Skerrit, Trisdikoon & Johnston 1981; Biggio, Concas, Mele & Corda, 1987; Bolden, Hambley, Johnston & Roger, 1990; Primus & Kellog, 1991; Martijena, Salvatierra & Arce, 1992). File, Andrews, Wu, Zharkovsky and Zangrossi (1992) have recently reported that handling can alter the behavioural and neurochemical effects of chlordiazepoxide in rats. It is possible that some of the apparent discrepancies in the BDZ tolerance and dependence literature are due to differences in environmental factors between different studies (File, 1982a; File, 1983). Similar environmental factors may partially explain the variability in the response of humans to BDZs (Woods, Katz & Winger, 1992). The effects of prior behavioural experience will be an important area of investigation for future animal studies of BDZ tolerance and dependence which may have important clinical implications.

Implications for future animal studies

Clearly, the clinical relevance of the animal literature on BDZ tolerance and dependence is reduced by the common use of high doses of BDZs, administered by routes and according to schedules which are quite unlike those used in humans. Due to species differences in the metabolism of BDZs, inevitably there will be limitations on the extent to which BDZ administration in rodents can be used as a model of BDZ therapy in humans. However, if future animal studies use lower, divided doses of BDZs, administered orally or by another route subject to first-pass metabolism, this would at least reduce the differences between basic experimental work in animals and the clinical application of BDZs in humans.

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