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Differentiating the sedative, psychomotor and amnesic effects of benzodiazepines: a study with midazolam and the benzodiazepine antagonist, flumazenil

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Abstract. Sixteen healthy volunteers were administered midazolam followed by placebo or the benzodiazepine antagonist, flumazenil, in a double-blind, cross-over study. Flumazenil reversed midazolam-induced sedation on the subjective, psychophysiological and motor indices used. In contrast, there was little evidence of any reversal of amnesic effects, which were assessed using both direct (explicit) and indirect (implicit) measures of memory. Results are discussed in terms of dissociating the sedative and amnesic effects of benzodiazepines.

Key words: Flumazenil – Midazolam – Memory – Sedation – Psychomotor impairment

All benzodiazepines in acute dosage can produce sedation, psychomotor impairments and anterograde amnesia (Curran 1986). However, the extent to which these effects are interdependent is still unclear, and this interdependence itself may vary with different benzodiazepines. The effects of benzodiazepines are thought to be mediated by facilitation of central GABA-ergic transmission via specific benzodiazepine receptors (Haefely 1985; Morre et al. 1985). If all the effects of benzodiazepines are mediated in this way, then administration of a benzodiazepine antagonist should reverse their sedative, psychomotor and amnesic effects.

Flumazenil is a specific benzodiazepine antagonist which has been shown in animal and human studies to reverse the sedative, muscle-relaxing, anxiolytic and anticonvulsant effects of benzodiazepines (Hunkheler et al. 1981; Darragh et al. 1982; Dorow and Duka 1986). However, the effect of flumazenil on benzodiazepine-induced memory impairments is less clear. O'Boyle et al. (1983) reported that flumazenil attenuated, but did not reverse, the amnesic effects of diazepam. Hommer et al. (1986) found that pretreatment with flumazenil blocked the sedative, anxiolytic and attentional effects of diazepam but not its amnesic effects. Dorow et al. (1987) found that the amnesic effects of lormetazepam were completely reversed by flumazenil. Dunton et al. (1988) reported differential dosage effects of flumazenil on the amnesic actions of diazepam as compared with lorazepam. Ricou et al. (1986) and Sage et al. (1987) assessed the effects of flumazenil on midazolam-induced sedation with patients undergoing surgical procedures. Results in terms of memory effects in both these studies are difficult to interpret for methodological reasons (Ricou et al. assessed amnesia as simply "present or absent" according to unclear criteria; Sage et al. presented the same stimuli on two different testing occasions).

In a previous study, we compared the effects of flumazenil compared with a placebo on 44 patients who had been given midazolam prior to day-care urological surgery (Birch and Curran 1990). We found that flumazenil reversed the psychomotor impairments induced by midazolam but not its amnesic effects. The present study was designed to assess first, whether these results could be replicated in a study with healthy, volunteer subjects, and second, to incorporate a wider range of indices of both sedation and memory. The test battery was selected to assess amnesic, psychomotor and sedative effects of the drugs. Sedation was assessed using subjective (rating scales), psychophysiological (critical flicker fusion threshold), and psychomotor (finger tapping) indices. Memory was assessed using both direct (free recall) and indirect (word-stem completion) measures.

Materials and methods

Subjects and design. Sixteen healthy volunteers (ASA grades I and II), nine men and seven women, were paid for participating in the study. All gave written, informed consent. Their ages ranged from 21 to 43 years (mean: 29) and their weight from 51 to 101 kg (mean: 70). They were not allowed alcohol or any other CNS drugs from 24 h before each test day.

A double-blind, cross-over design was used to compare the effects of intravenous flumazenil (0.5 mg in 5 ml) and placebo (inert carrier, 5 ml) in reversing the effects of intravenous midazolam. The

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Table 1. Group means and (in parentheses) standard errors

	Placebo				Flumazenil			
Testing times	T1	T2	T3	T4	T1	T2	T3	T4
Variable								
Tapping – N	353 (6)	287 (8)	306 (6)	324 (7)	351 (6)	287 (8)	331 (7)	337 (6)
CRT – ms	423 (10)	723 (44)	521 (23)	478 (21)	423 (11)	770 (38)	433 (14)	427 (13)
DSST – N	71 (1)	43 (3)	57 (3)	62 (2)	71 (2)	45 (3)	64 (2)	68 (2)
CFFT – Hz	48.2 (1.1)	39.2 (1.3)	42.5 (1.1)	45.5 (1.1)	47.4 (1.1)	38.8 (1.3)	46.5 (0.9)	47.2 (1.0)
Mood factor 1 (alertness) mm	36 (3)	66 (3)	66 (4)	56 (3)	38 (4)	64 (3)	52 (4)	42 (4)
Mood factor 2 (contentedness) mm	30 (4)	34 (4)	30 (4)	29 (4)	31 (3)	31 (4)	28 (4)	29 (3)
Mood factor 3 (calmness) mm	40 (5)	25 (3)	22 (4)	29 (4)	42 (6)	22 (3)	22 (2)	37 (3)
Delayed recall – N				6.3 (0.6)				7.2 (0.7)
Recognition								
A^1 index				−0.53 (0.30)				−0.32 (0.15)
B				−0.46 (0.07)				−0.43 (0.06)
Word completion of targets – N	5.8 (0.7)		3.6 (0.5)		6.3 (0.6)		4.2 (0.4)	

dosage of midazolam was titrated at the rate of 1–2 mg per min for each subject to achieve conscious sedation (such that speech was slurred and ptosis evident) and was the same for each individual on the 2 testing days. This dosage varied between 4 and 11 mg (0.06–0.14 mg · kg^{-1}: mean 0.10 mg · kg^{-1}). A minimum wash-out period of 2 weeks separated the two testing sessions.

Each of the two test days began with a practice session to familiarize each subject with the main assessments used (see below). Subjects were tested before the administration of midazolam (T1), again 15 min after sedation (T2) and then given flumazenil or placebo. Ten minutes later, subjects were re-tested (T3), and again, 60 min later (T4). All test versions were balanced across drug conditions using a Latin square.

Test battery. Finger tapping speed was determined by the number of key presses made in 60 s (Frith1967). *Choice reaction times* (CRT) were assessed using a computerised system in which 44 target stimuli were presented on a VDU (Wesnes et al. 1987). The subject's task was to press a black (left hand) or a red key (right hand) according to whether the target stimulus in the centre of the screen was the word "LEFT" or "RIGHT". Responses occurring in less than 200 ms were excluded as "anticipatory" and only reaction times for correct responses were included in the analysis. The *digit symbol substitution test* (DSST) was carried out for 90 s (Wechsler 1955). *Critical flicker fusion threshold* (CFFT) was assessed as described by Wesnes et al. (1987). Subjective ratings were made on the visual analogue *Mood Rating Scale* (MRS) which consists of 16 100 mm lines. Factor analysis of this scale yields three mood factors: alertness, contentedness and calmness (Bond and Lader 1974). All the above assessments were administered at each testing time.

Memory tasks were designed to assess retention both directly and indirectly. The main direct task was a verbal free recall task used in our previous study (Birch and Curran 1990). Sixteen bisyllabic nouns, matched across versions for word frequency, were presented for *immediate free recall* at each testing time. In addition, after the last testing session, subjects were asked for *delayed* recall of all words presented that day, and then given a yes-no *recognition* task which comprised words from all the four lists presented randomly mixed with an equal number of distractor words from matched lists not previously presented.

The indirect assessment of memory was administered only at the beginning of testing times T1 and T3. Subjects were shown a series of 20 words in a booklet, and asked to read aloud each word and rate how much they liked or disliked it on a 5-point scale (1 = dislike extremely; 5 = like extremely). Sixteen words were "target" words, and the other four words were fillers at the beginning and end of the booklet which were used to reduce primacy and recency. After the DSST and tapping tests, subjects were then given an ostensibly unrelated task which was to complete a series of three-letter word-stems with the first word that came to mind. There was no time limit for this task but subjects were asked to complete all the word-stems as quickly as possible. Each of the 24 stems could begin at least a dozen common English words listed in the Oxford MiniDictionary. However, 16 of the stems began target words previously rated for liking. The order of testing was the same for all subjects and all test periods.

Analysis of results. A multivariate analysis of variance (subjects, drugs, testing times) was carried out on all variables. Where specific comparisons at one testing time were needed, separate analyses of variance were performed (subjects, drugs). Recognition data were subjected to signal detection analysis using the method described by Frey and Colliver (1973). Means and standard errors of means are given in Table 1.

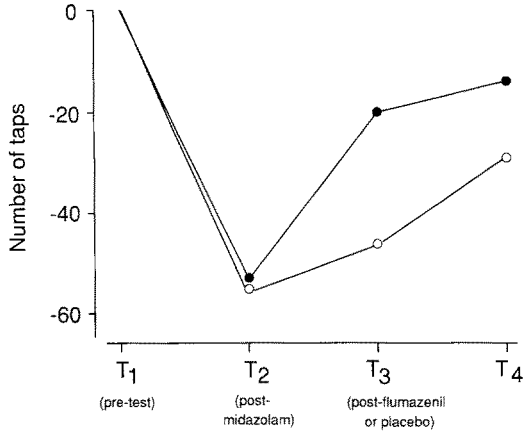

Fig. 1. Mean change from baseline in number of taps 60 s by subjects given placebo (○) or flumazenil (●)

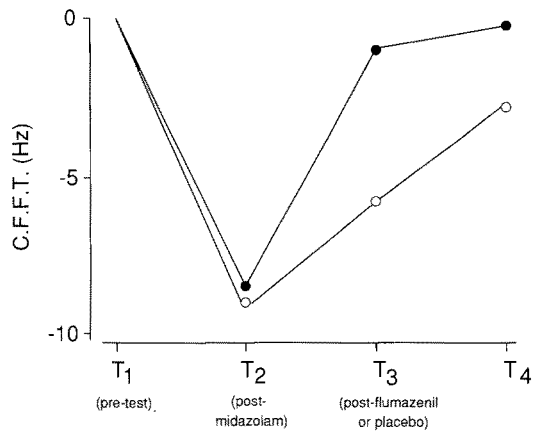

Fig. 3. Mean change from baseline in CFFT by subjects given placebo (○) or flumazenil (●)

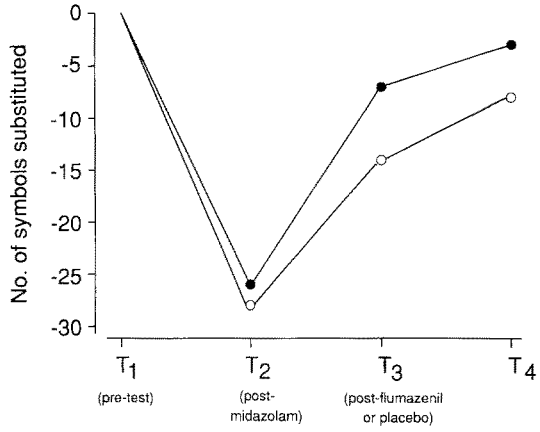

Fig. 2. Mean change from baseline in DSST performance by subjects given placebo (○) or flumazenil (●)

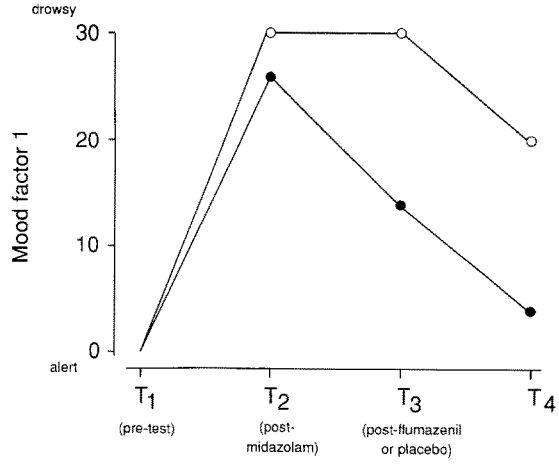

Fig. 4. Mean change from baseline in subjective ratings of alertness (mm) by subjects given placebo (○) or flumazenil (●)

Results

Finger tapping speed was markedly reduced after midazolam. However, this impairment was decreased by flumazenil compared with placebo, giving a significant drugs × testing times interaction [$F(3,16)=6.3$, $P<0.01$] (Fig. 1).

Choice reaction times showed a similar pattern of results [drugs × testing times: $F(3,12)=9.96$, $P<0.001$].

Digit symbol substitution test performance also showed a marked impairment following mindazolam which was significantly reversed by flumazenil compared with placebo [drugs × testing times: $F(3,12=8.3$, $P<0.005$] (Fig. 2).

Critical flicker fusion threshold depression was also significantly reversed by flumazenil, and returned almost to baseline values at T3 (Fig. 3) [drugs × testing times, $F(3,12)=26.0$, $P<0.001$].

Mood ratings. Mood factor 1 (alertness) revealed a significant difference between flumazenil and placebo (Fig. 4). Subjects rated increased drowsiness at T2 following administration of midazolam which persisted to T3 for placebo treatment. This subjective sedation was partially reversed by flumazenil at T3 and nearly fully reversed at T4, leading to a significant drugs × testing times interaction [$F(3,12)=8.5$, $P<0.005$]. There were no significant effects of flumazenil on the other two mood factors (contentedness, calmness).

Immediate word recall. Subjects were impaired on this task following administration of midazolam (Fig. 5). The MANOVA did not reveal any significant differences between flumazenil and placebo on this task. An ANOVA was then carried out at each testing time point separately (using change scores from baseline). This revelaed a significant difference between flumazenil and placebo at T3 only [$F(1,14)=9.1$, $P<0.01$].

Delayed word recall and recognition. No significant differences between flumazenil and placebo emerged from the analysis.

Indirect assessment of memory. The number of target words completed appeared reduced after midazolam (Fig. 6) but there was no significant difference between placebo and flumazenil treatments.

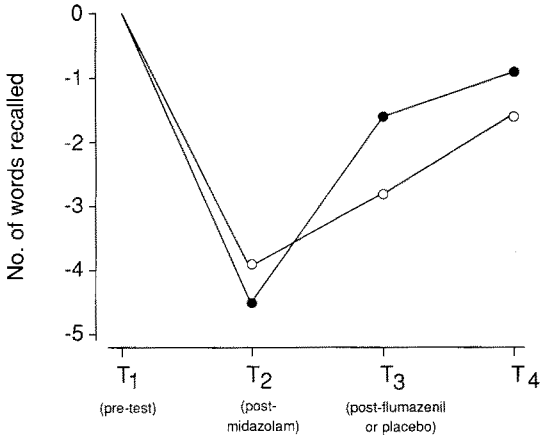

Fig. 5. Mean change from baseline in number of words recalled by subjects given placebo (○) or flumazenil (●)

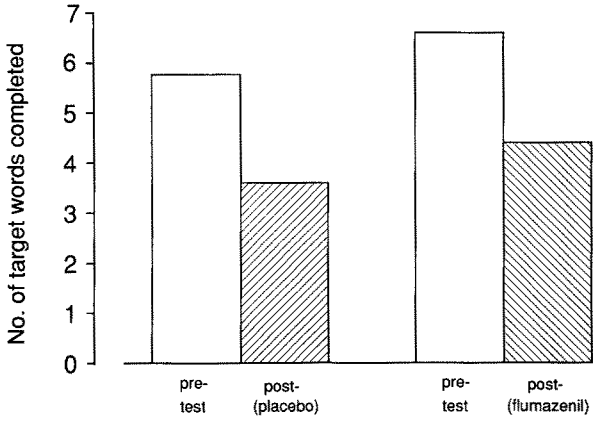

Fig. 6. Number of target words completed before drug administration and after flumazenil or placebo

Discussion

The assessment of sedation in this study was comprehensive, using all three putative indices (subjective ratings, motor speed and central "arousal"). Often, these three indices do not intercorrelate well and it has been argued that they tap different aspects of what is globally called "sedation" (e.g. Eysenck 1984). Our results showed clearly that flumazenil reverses the sedative effects of midazolam on all these indices of sedation.

Midazolam-induced sedation was wearing off slowly over the testing times, but between 10 min and 1 h after its administration, flumazenil significantly reduced sedation in terms of subjective ratings, CFFT and motor (tapping) speed. These findings thus replicate our previous findings with patients undergoing day-care surgery (Birch and Curran 1990).

Similarly, flumazenil reversed the psychomotor/attentional impairments induced by midazolam on the choice reaction time task, and the impairments of DSST, again replicating our findings with patients (Birch et al. 1990; Birch and Curran 1990).

In contrast to these very clear effects of flumazenil on sedative and psychomotor indices, our results do not provide any convincing evidence that flumazenil reverses the amnesic effects of midazolam. Our main analysis was used because it is most appropriate for repeated-measures data (MANOVA). This analysis did not reveal any significant differences between flumazenil and placebo on any memory variables. We carried out a subsequent ANOVA only to present our results in a very comprehensive way. That an ANOVA showed a significant improvement in immediate free recall 10–20 min after administration of flumazenil (compared with placebo) can only be seen as a marginal effect. ANOVA ignores the repeated measures nature of our data and analyses differences at each time point separately. This increases the likelihood of significant differences arising by chance, such that the null hypothesis is rejected when it should have been accepted (i.e. a type I error). In that context, our main conclusions must be based on the MANOVA findings showing no difference between flumazenil and placebo on immediate free recall. Further, the other two direct measures of memory used in this study, delayed recall and recognition, also revealed no evidence of reversal by flumazenil.

The results of the present study therefore parallel the results of our previous patient study which showed a clear reversal of the sedative and psychomotor effects of midazolam but not its (directly assessed) amnesic effects.

Memory assessed by direct measures such as recall or recognition is generally referred to as episodic (Tulving 1972) or explicit (Graf et al. 1984) memory. In contrast, indirect measures such as the word-stem completion task used in the present study are seen as tapping procedural or implicit memory. Evidence that the direct and indirect memory tasks may tap two separable memory systems derives mainly from studies showing that amnesic patients may show intact ("normal") performance on indirect tasks despite profound impairments on direct tasks. Little is yet known about the effects of psychotropic drugs on implicit memory. Two studies have shown that, although diazepam impairs performance on direct tests of memory, performance on indirect tests is not affected (Fang et al. 1987; Danion et al. 1989). This contrasts with a study by Brown et al. (1989) where the more potent benzodiazepine, lorazepam, was found to produce impaired performance on an indirect measure of memory (word-stem completion). The task was used here speculatively on the basis that midazolam, also a potent benzodiazepine, may impair retention as assessed by word-stem completion and that flumazenil may reverse this impairment. The results showed that there was no difference between flumazenil and placebo. Our data did indicate that subjects completed fewer target words after (as compared with before) midazolam. However, as this aspect of the study was not placebo controlled we cannot draw clear conclusions about the effects of midazolam per se on implicit memory. The possibility that different benzodiazepines may vary in their effects on implicit memory is nonetheless an intriguing one which we are currently exploring in another study.

There are several reasons why flumazenil may reverse psychomotor and sedative effects but have only a marginal effect on memory impairments. For instance, it may be that midazolam's sedative/psychomotor effects are not mediated by the same benzodiazepine receptor

complex which modulates memory. If this is true, it implies that there is more than one type of benzodiazepine receptor. Alternatively, it may be that the dose of flumazenil used (0.5 mg) was sufficient to reverse sedation but too low to reverse higher cognitive functions such as memory. Although we cannot discount this possibility, it is relevant to note that O'Boyle et al. (1983) used a massive, 200 mg dose of flumazenil and also found a differential sequence with diazepam (reversal of sedation but only attenuation of amnesic effects). It is further possible that flumazenil reverses the amnesic effects of midazolam but only for a brief time which occured before our 10-min testing point. In a study of dental anaesthesia using midazolam, Rosenbaum and Hooper (1988) report an improvement at 5 min after flumazenil compared with placebo on a simple word recognition task. Lastly, it is also possible that differential effects on sedation and memory reflect differential sensitivities of the measures used.

A central issue for those researching benzodiazepine-induced amnesia is the extent to which benzodiazepines have any specific amnesic effects which are not simply byproducts of their sedative effects. The relation between sedation and amnesia may vary across different benzodiazepines. However, as discussed in detail elsewhere (Curran 1991), there are now three lines of evidence indicating that benzodiazepines may have specific amnesic effects over and above sedative effects. Firstly, tolerance over repeated dosing builds up to sedative effects but residual memory impairments are still evident (Ghoneim et al. 1981). Secondly, two different benzodiazepines may produce similar sedative and psychomotor effects but different degrees of memory impairments (Curran et al. 1987). Thirdly, a benzodiazepine antagonist may reverse sedation but not fully reverse the amnesic effects of a benzodiazepine (e.g. Hommer et al. 1986; Birch and Curran 1990). The present study thus provides some further evidence for a degree of dissociation between the amnesic and sedative effects of midazolam.

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References

Birch BRP, Curran HV (1990) The differential effects of flumazenil on the psychomotor and amnesic actions of midazolam. J Psychopharmacol 4:29–34

Birch BRP, Anson KM, Gellister J, Parker C, Miller RA (1990) The role of midazolam and flumazenil in urology. Acta Anaes Scand [Suppl. 92] 34:25–32

Bond AJ, Lader MH (1974) The use of analogue scales in rating subjective feelings. Br J Med Psychol 47:211–218

Brown MW, Brown J, Bowes J (1989) Absence of priming coupled with substantially preserved recognition in lorazepam induced amnesia. Q J Exp Psychol 41A:599–617

Curran HV (1986) Tranquillising memories: a review of the effects of benzodiazepines on human memory. Biol Psychol 23:179–213

Curran HV (1991) Antianxiety treatments and human memory In: Brierly M, File S (eds) New concepts in anxiety. McMillan, London

Curran HV, Schiwy W, Lader M (1987) Differential amnesic properties of benzodiazepines: a dose response comparison of two drugs with similar elimination half-lives. Psychopharmacology 92:358–364

Danion JM, Zimmermann M-A, Willard-Schroeder D, Grange D, Singer L (1989) Diazepam induces a dissociation between explicit and implicit memory. Psychopharmacology 99:238–243

Darragh A, Lambe R, Kenny M, Brick I, Taafe WO, O'Boyle C (1982) Ro 15–1788 antagonises the central effects of diazepam in man without altering diazepam availability. B J Clin Pharmacol 14:677–682

Dorow R, Duka T (1986) Anxiety: its generation by drugs and their withdrawal. In: Biggio C, Costa E (eds) GABAergic transmission and anxiety. Raven Press, New York, pp 221–225

Dorow R, Berenberg D, Duka T, Sauerberg N (1987) Amnestic effects of lormetazepam and their reversal by the benzodiazepine antagonist Ro 15–1788. Psychopharmacology 93:507–514

Dunton AW, Schwam E, Pitman V, McGrath J, Hendler J, Siegal J (1988) Flumazenil: US clinical pharmacology studies. Eur J Anaethesiol 52:81–95

Eysenck MW (1984) A handbook of cognitive psychology. Lawrence Erlbaum, London

Fang JC, Hinrichs JV, Ghoneim MM (1987) Diazepam and memory: evidence for spared memory function. Pharmacol Biochem Behav 28:347–352

Frey PW, Colliver JA (1973) Sensitivity and responsivity measures in discrimination learning. Learn Motiv 4:327–342

Frith CD (1967) The effects of nicotine on tapping. Life Sci 6:321–326

Ghoneim MM, Mewaldt SP, Berie JL, Hinrichs JV (1981) Memory and performance effects of single and 3 week administration of diazepam. Psychopharmacology 73:147–151

Graf P, Squire LR, Mandler G (1984) The information that amnesic patients do not forget. J Exp Psychol Learn Mem Cogn 10:164–178

Haefely W (1985) Tranquillizers. In: Grahame-Smith DG, Cohen PJ (eds) Psychopharmacology 2, Part 1: Preclinical psychopharmacology. Elsevier, Amsterdam

Hommer DW, Brier A, Paul SM, Davis M, Weingartner H (1986) Ro 15–1788, a specific benzodiazepine antagonist, blocks the sedative, anxiolytic and attentional but not the amnestic effects of diazepam in humans. Abstracts of the 25th ANCP Annual Meeting, Washington

Hunkeller W, Mohler H, Pieri L, Polc P, Bonetti E, Cumin R, Schaffner R, Haefly W (1981) Selective antagonists of the benzodiazepines. Nature 290:574–576

Morre M, Bachy A, Keane PC (1985) Differential CNS binding of benzodiazepines. Supplement Actualites Medicales Internationales: Psychiatrie 2:28–32

O'Boyle C, Lambe R, Darragh A, Taffe W, Brick I, Kenny M (1983) Ro 15–1788 antagonises the effects of diazepam in man without affecting its bioavailability. Br J Anaesth 35:349–354

Ricou B, Forster A, Bruckner P, Chastonay P, Gemperle M (1986) Clinical evaluation of a specific benzodiazepine antagonist (Ro 15–1788). Br J Anaesthesiol 58:1005–1011

Rosenbaum NL, Hooper PA (1988) The effects of flumazenil, a new benzodiazepine antagonist, on the reversal of midazolam sedation and amnesia in dental patients. Br Dent J 165:400–402

Sage DJ, Close A, Boas RA (1987) Reversal of midazolam sedation with anexate. Br J Anaethesiol 59:459–464

Tulving E (1972) Episodic and semantic memory. In: Tulving E, Donaldson W (eds) Organisation of memory. Academic Press, New York, pp 381–403

Wechsler D (1955) Manual for the Wechsler Adult Intelligence Scale. Psychological Corporation, New York

Wesnes K, Simpson PM, Christmas L (1987) The assessment of human information processing abilities in psychopharmacology. In: Hindmarch I, Stonier PD (eds) Human psychopharmacology: measures and methods, vol 1. Wiley, Chichester, pp 79–92