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The Effect of Diazepam on Indices of 5-HT Function in Man

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NUTT, D. J., P. J. COWEN, M. FRANKLIN, P. MURDOCK, B. GOSDEN AND S. FRASER. *The effect of diazepam on indices of 5-HT function in man.* PHARMACOL BIOCHEM BEHAV **24**(5) 1491–1495, 1986.—The effects of acute and chronic diazepam administration on L-tryptophan induced prolactin release was studied in seven male volunteers. Acute diazepam diminished the prolactin neuroendocrine response to L-tryptophan. On chronic administration this effect was lost, suggesting tolerance had developed. The sedative effects of L-tryptophan were unaltered by either acute or chronic diazepam administration. A possible explanation for the tolerance development to the neuroendocrine effects may be the observed reduction in platelet ^{3}H-imipramine binding that was observed.

L-Tryptophan Diazepam Prolactin ^{3}H-Imipramine Platelet Neuroendocrine challenge test 5-HT function

THE benzodiazepines (BDZs) are probably the most widely used psychotropic drugs worldwide. In the United Kingdom it has been estimated that over 250,000 persons have taken a BDZ daily for the past 7 years [18]. Estimates from the USA show a similar level of chronic use with 2.4 percent of the adult population using a BDZ daily for the previous year [15]. Despite the recent trend away from long-term prescribing, the problems of chronic BDZ usage seem likely to persist for many decades.

Such chronic usage is often associated with the development of tolerance to the effects of these drugs. This may most readily be seen in the recrudescence of seizures when BDZs are used for their anticonvulsant effects [10]. Tolerance development to the therapeutic anxiolytic effects is more controversial. However, what is established is that after many months or years of BDZ consumption a withdrawal syndrome is revealed on cessation of dosage in a proportion of patients [18,20]. Animal studies have suggested that changes in BDZ receptor number do not account for this [2,4], although some recent work in our group has suggested changes in BDZ receptor coupling may account for withdrawal phenomena [12].

Another possibility is that chronic administration of BDZs produces compensatory alterations in other neurotransmitter systems. Perhaps the most likely site for such a change is the GABA receptor (see [8]). However, alterations in monoamine function might also be implicated since for many years BDZs have been known to interact with these monoamine pathways (see this issue).

The animal evidence suggesting that BDZs interact with 5-HT pathways is reviewed elsewhere is this issue. There have been few studies in man, a fact which probably reflects the difficulties of assessing 5-HT function *in vivo*. The only direct evidence for an effect of BDZs on 5-HT metabolism comes from the study of Petursson *et al.* [17]. This showed that in a group of 4 patients who had been taking BDZs (mostly diazepam) for between 1 and 20 years urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) was increased significantly during their withdrawal from these drugs. Although the 5-HIAA levels pre-withdrawal were low they were still within the normal range. However, they more than doubled during the withdrawal period. This suggests that 5-HT turnover is relatively reduced during chronic BDZ use and returns to normal during withdrawal.

Recently attempts have been made to assess brain 5-HT function more directly. The tests employed have centered on the use of the neuroendocrine challenge model in which an increase in brain 5-HT function is followed by the release of prolactin and growth hormone [14]. In man three main pharmacological approaches have been used to increase brain 5-HT function: the 5-HT-releasing agent, fenfluramine [19], and the precursors, 5-hydroxytryptophan (5-HTP) [16] and L-tryptophan (LTP) [3]. Although from a theoretical viewpoint fenfluramine is probably the most selective and well defined challenge test [6] it is generally used orally and so takes many hours to produce its effects. This makes studies tedious for the subjects and of uncertain validity. In contrast LTP may be given intravenously, and produces a rapid release of prolactin that peaks at 45 min after the start of the infusion. A complete test may be performed in under 3 hours [7].

We have used the LTP challenge test to examine 5-HT function in man before and after 3 weeks' administration of a moderately high dose (25 mg/day) of diazepam. In addition the effect of a single acute dose of 15 mg was subsequently studied.

Finally, we also assessed the effect that diazepam treat-

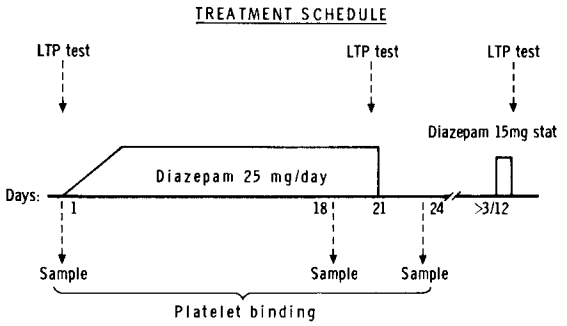

FIG. 1. Experimental protocol for study showing timing of tryptophan tests and platelet receptor binding.

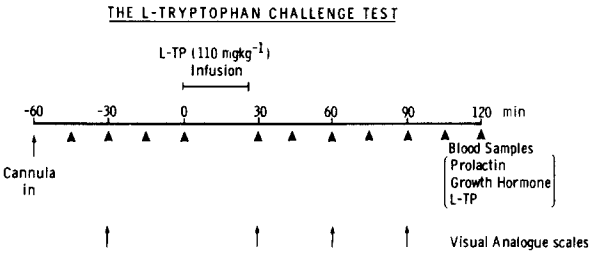

FIG. 2. Time course of an individual L-tryptophan test.

ment had on a peripheral index of 5-HT function; [^{3}H]-imipramine binding in platelets. This ligand labels a part of the 5-HT uptake system [11] and shows very similar binding characteristics in platelets as in brain. Consequently it has been suggested that imipramine binding changes may mirror central changes in 5-HT uptake [11].

METHOD

Subjects

Eight male volunteers (aged 20–35 years) who had given informed consent were recruited into the study. They were physically fit and had full biochemical and haematological assessment prior to entry. None were taking any medication. Only 1 used tobacco and none were heavy drinkers. One subject dropped out of the study due to personal difficulties and his results have been excluded. The study was approved by the local Psychiatric Sector Research Ethics Committee.

Drugs

Diazepam (Roche) was administered orally in 5 mg tablets. Ideally subjects would have been given a loading dose of both diazepam and its main metabolite, desmethyl diazepam, and then a maintenance dose of 25 mg/day. However this would have produced profound and possible dangerous sedation in the early stages. To minimise the deleterious effects of the drug regime a gradually increasing dosage was used so that the final dose of 25 mg/day (as 10 mg in the morning, 5 mg at lunch-time and 10 mg at night) was attained by day 6 (see Fig. 1). This dose was then maintained until day 21. In order to assess the effects of an acute high dose subjects were given a washout period of at least 3 months and then took a single 15 mg dose of diazepam the night before their 3rd LTP test.

LTP testing was performed as detailed in [7]. Briefly LTP powder (E. Merck) was dissolved in 0.45% saline and sterilized by passage through an 0.22 μm Millipore filter. A pre-warmed solution was administered IV at a dose of 110 mg/kg (100 mg/ml) through an intravenous cannula.

On test days subjects fasted from midnight (though took their a.m. diazepam if required. LTP infusions were carried out in the Research Unit starting at 09.00 hr. Subjects were tested reclining, and were not allowed to sleep.

Blood samples were taken at the times shown in Fig. 2. In addition, at t=−30 a 45 ml sample for plasma diazepam and platelet receptor binding was withdrawn.

The sedative effects of the LTP infusion were assessed with a 100 mm visual analogue scale (sleepiness rating scale). This was arranged so that a score of 0 represented "not at all sleepy" and one of 100 "the most sleepy I have ever been." The scale was administered at the times shown in Fig. 1.

Assays

Plasma prolactin was assayed by standard double antibody radioimmunoassay. The average intra-assay and inter-assay coefficients of variation were 6.7% and 8.8%. Prolactin responses were assessed by measuring the area under the curve (Simpson's rule) for t=0–120 and subtracting extrapolated baseline (t=−0) readings.

Plasma diazepam and desmethyl diazepam concentrations were assayed by a high performance liquid chromatographic (HPLC) technique following a solvent extraction from alkalinised plasma. The HPLC system consisted of a Constrametric I (Laboratory Data Control, Stone, U.K.) twin-pistoned HPLC pump; a Cecil Model 212 UV detector (Cecil Instruments, Cambridge, U.K.), a Rheodyne 7125 injection valve fitted with a 50 μl loop and a Brownlee HPLC cartridge pack containing a 10 cm reverse-phase 5 μM ODS analytical column protected by a similar 3 cm guard column. The solvent system consisted of 0.03 M phosphate buffer pH 7.8 and methanol (in the ratio 3:7). Tube flow rate was 1 ml per minute and the wavelength for the UV detector was net at 215 nM. The inter-assay and intra-assay coefficients of variation were respectively 12.5% and 8%.

Platelet [^{3}H]-imipramine binding was performed according to the method described in [1]. Whole platelets separated by centrifugation were washed and resuspended in 0.1% EDTA. The suspension was incubated with [^{3}H]-imipramine over a concentration range of 0.5–5 nM at 4° for 60 min and then terminated by centrifugation. Non-specific binding was determined by displacement with fluoxetine at a final concentration of 1 μM.

RESULTS

LTP Test—Prolactin Responses

The dose of LTP used in this study produces a robust rise in plasma prolactin. After three weeks of diazepam administration prolactin responses were not significantly different from the pre-treatment values (Figs. 3a and 3b). In contrast the acute single dose of diazepam produced a marked fall in prolactin response to LTP. This did not quite reach significance when compared with the pre-treatment AUC but LTP response was significantly reduced when compared with the

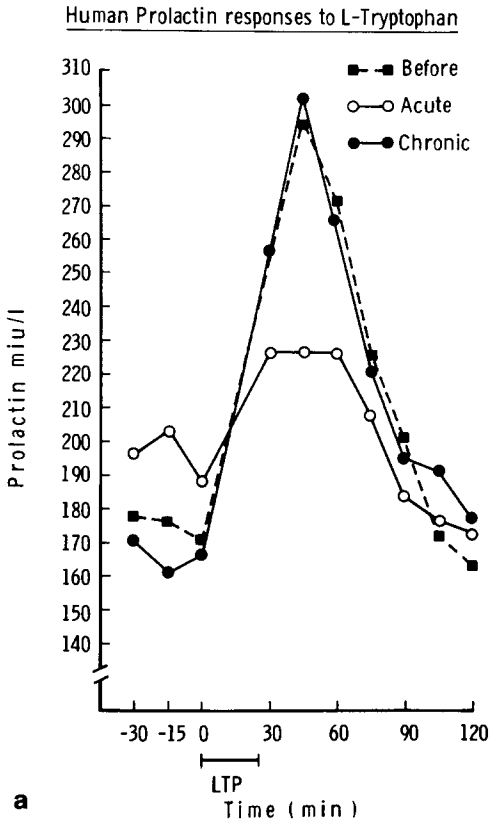

FIG. 3a. Time course of prolactin response to LTP before diazepam, following chronic and acute diazepam administration.

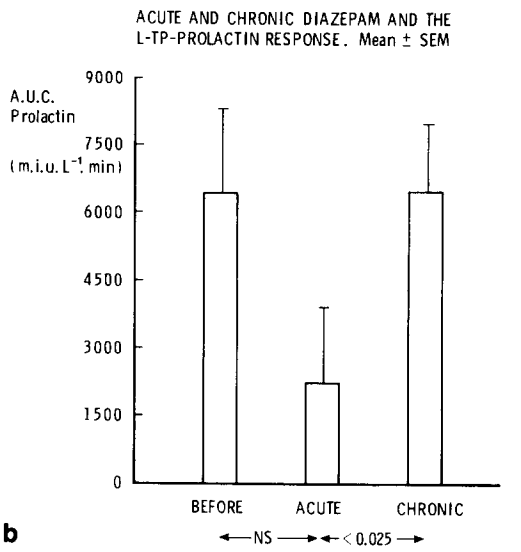

FIG. 3b. Area under the curve LTP-prolactin responses before diazepam and following acute and chronic diazepam administration. Histograms represent mean±S.E.M.

TABLE 1

LTP-PROLACTIN RESPONSES: INDIVIDUAL VALUES

Pre	Acute	Chronic
8910	1785	5610
9225	1425	7515
1770	315	5490
150	0	0
3825	12060	13545
15975	0	6075
9240	0	0
7020 ± 2055	2220 ± 1665	6375 ± 1785

Individual prolactin responses (AUC: miU·L^{-1}·min). 0=No response.

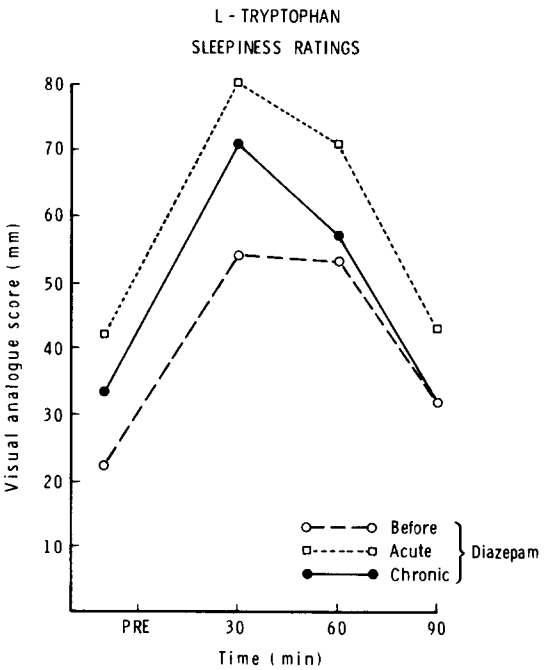

FIG. 4. Changes in self-rated sleepiness scores produced by L-tryptophan in the 3 test sessions.

responses during chronic diazepam administration (Figs. 3a and 3b). Individual values are given in Table 1.

Sedative Responses

As can be seen from Fig. 4, the LTP infusion produces a transient increase in self-rated sleepiness with a peak at +30. Neither the chronic nor the acute diazepam produced a significantly different peak response (Fig. 4). The apparent differences are due to the somewhat elevated baseline (pre-LTP) levels of sleepiness when on drug. However none of the three baseline (pre-LTP) points differ as the range of individual scores is large (before: 10–60, acute: 0–70, chronic: 0–60). Both acute and chronic vs. before $p>0.1$, Mann Whitney U-test.

Platelet Imipramine Binding

During chronic diazepam administration a small but consistent fall in B_{max} was observed (Fig. 5). This was significant

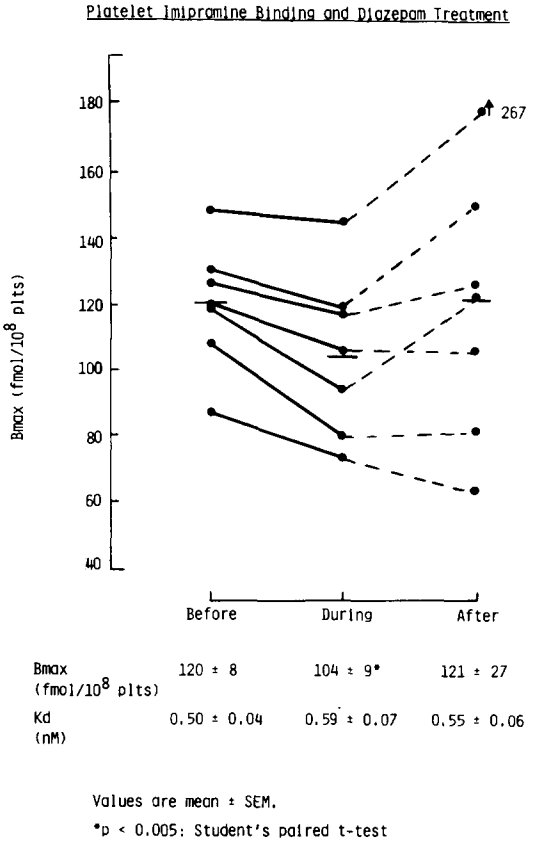

FIG. 5. Changes in [^{3}H]-imipramine binding to platelets of individual subjects before diazepam, after chronic diazepam, and during the phase of diazepam washout after chronic administration. The inset to the figure gives mean B_{max} values±S.E.M. as well as mean K_d values±S.E.M. for the 3 binding periods.

($p<0.005$) on a paired t-test. After cessation of drug binding returned to pre-diazepam values. Binding was not performed after the single acute dose of diazepam as we have previously shown that benzodiazepines do not directly interfere with platelet [^{3}H]-imipramine binding (K. Stump, unpublished observations).

Plasma Drug Levels

These are shown in Fig. 6. As expected, plasma levels of both diazepam and its metabolite, desmethyl diazepam, were greater during chronic administration than following the acute dosage. In particular, the levels of the long-acting metabolite, desmethyl diazepam, were increased.

DISCUSSION

These findings show that following the acute administration of a moderate dose (15 mg) of diazepam the prolactin response to LTP is reduced as compared with no drug and chronic diazepam tests. Although the comparison with the former test does not reach statistical significance in the group of 7 subjects, there was a pronounced fall in the responses of 6. Further work is in progress to confirm this finding. All subjects had greater responses in the chronic phase than in the acute. This occurred despite markedly elevated levels of plasma diazepam and metabolite and so suggests a functionally significant adaptive change or tolerance to the acute effects of BDZs.

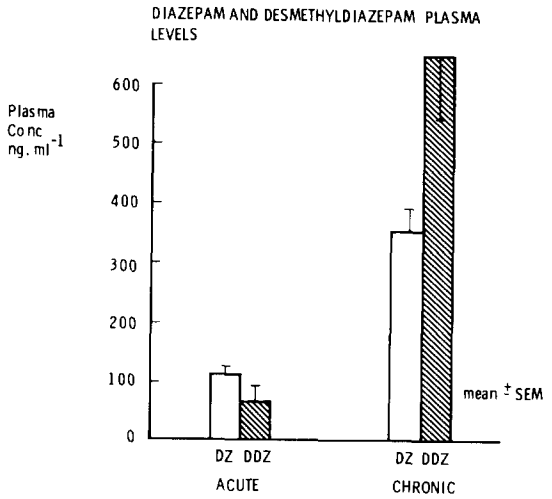

FIG. 6. Plasma levels of diazepam and its main metabolite, desmethyl diazepam, measured at the time of the acute and the chronic LTP tests.

The lack of change in the sleepiness produced by LTP is hard to interpret. This may suggest that the systems mediating this are different to those involved in the prolactin change. However, the fact that the sedative effects of diazepam may add in to those of LTP makes interpreting sedation data difficult. At present there is good evidence that the prolactin response to LTP is mediated via 5-HT receptors [6]. It is less clear whether sedation is similarly mediated and it has been suggested that this might act via BDZ receptors [13]. However, if this were the case one might expect the administration of the high affinity BDZ receptor ligand diazepam to prevent access of tryptophan to the receptor.

Possible explanations for the recovery of normal prolactin responses during chronic diazepam administration are increased 5-HT synthesis or enhanced post-synaptic receptor sensitivity. Animal evidence such as the increased head twitch response to 5-HTP and the increased 5-HT$_2$ receptor number in cortex following diazepam [9] supports the second possibility. The idea that tolerance develops to the BDZ-induced reduction in 5-HT turnover [5] is still controversial (see this issue).

The changes in platelet imipramine binding may, if similar ones occur in the brain, offer a possible explanation. The reduction of imipramine binding sites would be expected to result in reduced 5-HT uptake. This should lead to reduced synaptic clearance and thus might enhance post-synaptic responses to released 5-HT.

In conclusion, therefore, we have evidence in man that moderate doses of diazepam acutely inhibit the prolactin response to the putative 5-HT challenge test LTP. Sedative responses are unaltered. Despite very much higher plasma levels after 3 weeks' administration of diazepam the prolactin response returns to normal. It remains to be determined whether a further increase in prolactin response is seen in patients who have been on BDZs for very much longer periods. The possibility that some form of 5-HT overactivity or rebound might contribute to the phenomenon of BDZ withdrawal should be considered.

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REFERENCES

1. Braddock, L. E., P. J. Cowen, J. M. Elliott, S. Fraser and K. Stump. Changes in the binding to platelets of [^{3}H]imipramine and [^{3}H]yohimbine in normal subjects taking amitriptyline. *Neuropharmacology* **23:** 285–286, 1984.
2. Braestrup, C., M. Nielsen and R. F. Squires. No changes in rat benzodiazepine receptors after withdrawal from continuous treatment with lorazepam and diazepam. *Life Sci* **24:** 347–350, 1979.
3. Charney, D. S., G. R. Heninger, J. F. Renhard, D. E. Sternberg and K. M. Hafstead. Effect of intravenous L-tryptophan on prolactin and growth hormone and mood in healthy subjects. *Psychopharmacology (Berlin)* **77:** 217–222, 1982.
4. Chiu, T. H. and H. C. Rosenberg. Reduced diazepam binding following chronic benzodiazepine treatment. *Life Sci* **23:** 1153–1158, 1978.
5. Collinge, J., C. J. Pycock and P. V. Taberner. Studies on the interaction between cerebral 5-hydroxytryptamine and gamma-aminobutyric acid in the mode of action of diazepam in the rat. *Br J Pharmacol* **79:** 637–643, 1983.
6. Cowen, P. J. and I. M. Anderson. 5-HT neuroendocrinology: changes during depressive illness and antidepressant drug treatment. *Br J Psychiatry*, in press.
7. Cowen, P. J., H. Gadhvi, B. Gosden and T. Kolakowska. Responses of prolactin and growth hormone to L-tryptophan infusion: effects in normal subjects and schizophrenic patients receiving neuroleptics. *Psychopharmacology (Berlin)* **86:** 104–109, 1985.
8. Cowen, P. J. and D. J. Nutt. Abstinence symptoms after withdrawal of tranquillizing drugs: Is there a common neurochemical mechanism? *Lancet* **ii:** 360–362, 1982.
9. Green, A. R., P. Johnson, J. A. Mountford and V. L. Nimogoankar. Some anticonvulsant drugs alter monoamine-mediated behaviours in mice in ways similar to electroconvulsive shock; implications for antidepressant therapy. *Br J Pharmacol* **84:** 337–346, 1985.
10. Greenblatt, D. J., E. Wood, M. D. Allen, P. J. Orsulak and R. I. Shader. Rapid recovery from massive diazepam overdose. *J Am Med Assoc* **240:** 1872–1874, 1978.
11. Langer, S. Z. and R. Raisman. Binding of ^{3}H-imipramine and ^{3}H-desipramine as biochemical tools for studies in depression. *Neuropharmacology* **22:** 407–413, 1983.
12. Little, H. J., D. J. Nutt and S. C. Taylor. Selective potentiation of the effects of a benzodiazepine contragonist after chronic flurazepam treatment in mice. *Br J Pharmacol* **83:** 360P, 1984.
13. Marangos, P. J., J. Patel, F. Hirata, D. Sondheim, S. M. Paul, P. Skolnick and F. K. Goodwin. Inhibition of diazepam binding by tryptophan derivatives including melatonin and its brain metabolite N-acetyl-5-methoxykynureamine. *Life Sci* **29:** 259–267, 1981.
14. Meites, J. and W. E. Sonntag. Hypothalamic, hypophysiotropic hormones and neurotransmitter regulation: current views. *Annu Rev Pharmacol Toxicol* **21:** 295–322, 1981.
15. Mellinger, G. and M. B. Balter. Prevalence and patterns of use of psychotherapeutic drugs: results from a 1979 national survey of American adults. In: *Epidemiological Impact of Psychotropic Drugs*, edited by G. Tognoni, C. Bellantuono and M. Lader. Amsterdam: Elsevier/North Holland, 1981, pp. 117–135.
16. Meltzer, H. Y., B. Wiita, B. J. Tricou, M. Simonovic, V. Fang and G. Manov. Effect of serotonin precursors and serotonin agonists on plasma hormone levels. In: *Serotonin in Biological Psychiatry*, edited by B. M. Ho *et al.* New York: Raven Press, 1982.
17. Petursson, H., P. A. Bond, B. Smith and M. H. Lader. Monoamine metabolism during chronic benzodiazepine treatment and withdrawal. *Biol Psychiatry* **18:** 207–213, 1983.
18. Petursson, H. and M. H. Lader. *Dependence on Tranquillizers*. Oxford: Oxford University Press, 1984.
19. Quattrone, A., A. Tedeschi, U. Aguglia, F. Scopacasa, G. F. Direnzo and L. Annunziato. Prolactin secretion in man: a useful tool to evaluate the activity of drugs on central 5-hydroxytryptaminergic neurones: studies with fenfluramine. *Br J Clin Pharmacol* **16:** 471–475, 1983.
20. Tyrer, P., R. Owen and S. Dawling. Gradual withdrawal of diazepam after long-term therapy. *Lancet* **i:** 1402–1406, 1983.