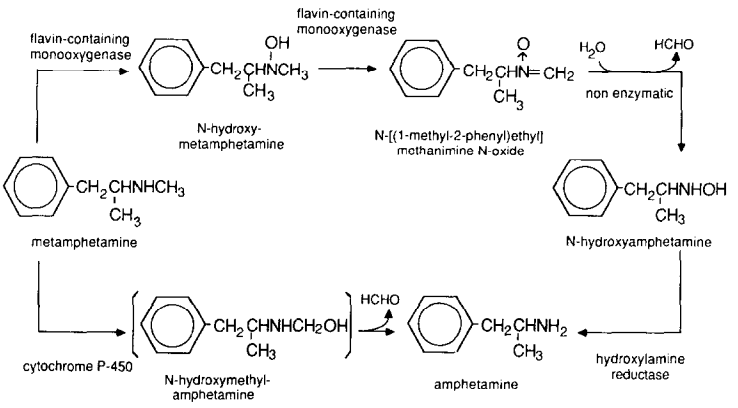


Fig. 1. Proposed metabolic pathway in *N*-demethylation of methamphetamine.

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# Benzodiazepine and $GABA_A$ receptors in rat brain following chronic antidepressant drug administration

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Chronic administration of antidepressant drugs to animals is associated with changes in the number and function of several classes of central monoamine receptors [1, 2]. For example, decreases in the number of cortical $\beta$-adrenoceptors and in the sensitivity of the associated noradrenaline-stimulated adenylate cyclase occur after chronic administration of all classes of antidepressant drugs and repeated electroconvulsive shocks [1–5]. Decreases in $5HT_2$ and $\alpha_2$ adrenoceptor number and function have also been reported although such effects are not seen with all antidepressant treatments [1, 2, 6–8]. Recently cerebral $\gamma$-aminobutyric acid (GABA*) receptor subtypes ($GABA_A$ and $GABA_B$) have been studied after chronic antidepressant drugs although the results as yet are inconsistent. Marked increases in $GABA_B$ binding sites in rat frontal cortex have been reported following 18 days administration of several antidepressant drugs by minipump infusion [9, 10]. No reproducible effects on $GABA_A$ receptor binding were found in these studies [9]. However, the numbers of high and low affinity $GABA_A$ binding sites were reduced in the mouse cortex and hippocampus following daily administration of imipramine or nomifensine for 14 days [11]. This effect of chronic administration of antidepressant drugs on $GABA_A$ receptors is supported by a reduction in benzodiazepine (BZ) binding sites [12], a component of the $GABA_A$–ionophore complex. The magnitude of this latter effect is surprising, with decreases of 58–75% in the number of binding sites after desmethylimipramine, zimelidine, buproprion and adinazolam (10 mg/kg twice daily for 21 days). We now report a study of chronic administration of three antidepressants on BZ binding sites in the rat brain. We have also measured concurrently the ability of GABA to stimulate BZ binding.

*Abbreviations used: GABA, $\gamma$-aminobutyric acid; BZ, benzodiazepine.

Table 1. Effect of chronic antidepressant administration on the binding of [$^3$H]flunitrazepam

| | $K_D$ (nM) | $B_{max}$ (pmoles/mg protein) |
|---|---|---|
| Saline control | 0.60 ± 0.08 | 1.29 ± 0.16 |
| Naïve control | 0.77 ± 0.16 | 1.18 ± 0.06 |
| Desmethylimipramine | 0.56 ± 0.08 | 1.13 ± 0.12 |
| Tranylcypromine | 0.64 ± 0.09 | 1.12 ± 0.06 |
| Zimelidine | 0.49 ± 0.05 | 1.09 ± 0.15 |

Values are means ± SE for 4 determinations.

This effect of GABA is dose dependent, bicuculline blockable and is considered to be a functional measure of $GABA_A$/BZ site coupling [13].

*Materials and methods*

Male Sprague–Dawley rats (initial weight 150–170 g) were injected i.p. twice daily (8–9 a.m. and 4–6 p.m.) for 21 days with desmethylimipramine (10 mg/kg), zimelidine (10 mg/kg), tranylcypromine (5 mg/kg) or saline (injected controls). A further group of age- and weight-matched rats were housed in parallel but were not handled (other than for cage cleaning) and received no injections (naïve controls). Animals were weighed every 2–3 days and the dose of drug adjusted. The volume of injection was maintained at 0.5 ml/dose throughout. The animals were killed by decapitation, 24 hr after the last injection and the brains frozen at −20°. To prepare the membranes, brains were thawed, homogenised in 30 vol. (w/v) 50 mM Tris–citrate pH 7.1 using a motor-driven Teflon pestle and centrifuged at 50,000 *g* for 10 min at 4°. The supernatant was discarded and the procedure repeated two further times. The crude membranes, suspended in buffer, were stored at −20° until assayed. On the day of assay, the membranes were thawed, homogenised and centrifuged (as above) five times. [$^3$H]Flunitrazepam (eight concentrations, 0.5–15 nM) was incubated with membrane (equivalent to 2.5 mg original wet wt) at 0° for 90 min in a volume of 1 ml. The effect of GABA (9 concentrations, 100 nM–15 μM in the presence of 200 mM NaCl) on the binding of 0.5 nM [$^3$H]flunitrazepam was studied. Specific binding was defined as the binding displaced by 2 μM clonazepam. Membrane-bound radioactivity was recovered by filtration under vacuum through Whatman GF/B filters using a Brandell cell harvester. Filters were washed with 16 ml ice-cold buffer and radioactivity determined by liquid scintillation counting at an efficiency of 40–45%. Protein was determined by the method of Lowry *et al.* [14] using bovine serum albumin as standard. The maximal number of binding sites ($B_{max}$) and equilibrium dissociation constant ($K_D$) were determined by linear regression analysis of Scatchard plots. For GABA enhancement of BZ binding the percentage stimulation (compared with no GABA) was plotted against log GABA concentration. Maximal stimulation and the GABA concentration causing half maximal stimulation ($EC_{50}$) were determined graphically.

*Results and discussion*

Desmethylimipramine, tranylcypromine and zimelidine added directly to the [$^3$H]flunitrazepam (0.5 nM) binding assay did not significantly affect binding at $10^{-5}$ M. At $10^{-4}$ M they caused 37%, 6% and 44% inhibition of binding respectively. Twenty-one days administration of the antidepressants did not significantly alter either the number or affinity of BZ binding sites, compared to saline-injected or naïve controls (Table 1). The largest difference was a 16% reduction in the number of binding sites after zimelidine. These results do not replicate, with respect to desmethylimipramine or zimelidine, the marked reductions in BZ binding sites previously reported [12]. Methodological factors are unlikely to explain these discrepancies since we have used the same strain, sex and weight of rats, the same dose, route and duration of drug administration and the same radioligand as Suranyi-Cadotte *et al.* [12]. We have used an extensively washed and freeze-thawed membrane preparation to maximise removal of administered drug and endogenous GABA. However, it is unlikely that this will explain the discrepancy since the antidepressants only influence BZ binding at concentrations above $10^{-5}$ M and it is doubtful if such concentrations of drug would be present even in the less well-washed membranes used by Suranyi-Cadotte *et al.* [12]. Similarly the presence of endogenous GABA is unlikely to provide the explanation since GABA increases the affinity of BZs for their binding sites without altering the number of sites [15].

In the present study the number of BZ binding sites in whole brain membranes from control rats was about 1.2 pmoles/mg protein. This value is predictably lower than the 1.6–2.1 pmoles/mg protein which we have previously reported for cerebral cortex from male Wistar rats [16], but is considerably greater than the number of sites (0.35 pmoles/mg protein) reported by Suranyi-Cadotte *et al.* [12]. Several endogenous peptides, including diazepam binding inhibitor (DBI) and an octadecaneuropeptide derived from DBI [17–19], have been shown to be present in rat brain and to displace specific BZ binding. It is possible that the low control binding of Suranyi-Cadotte *et al.* [12] is a result of less than complete removal of such substances. Similarly the effects of antidepressants might be mediated via alterations in the concentration of such substances since we find no evidence for an effect on the BZ binding sites themselves.

In this study, rather than measuring GABA receptors by radioligand binding, we have chosen a functional measure, the ability of GABA acting on $GABA_A$ receptors to stimulate BZ binding. No differences were found in either the $EC_{50}$ value or the maximal stimulation following chronic antidepressant drug administration (Table 2). These results provide function evidence to support the findings of Pilc and Lloyd [9] but differ from those of Suzdak and Gianutsos [11]. However, the latter authors used different drugs to those used in our study and performed the experiments in mice rather than rats. Antidepressant drugs may differ in their ability to induce changes in $GABA_A$ receptor binding or the binding sites that are altered may not be functionally involved in the response we have measured.

Table 2. Effect of chronic antidepressant administration on GABA stimulation of [$^3$H]flunitrazepam binding

| | $EC_{50}$ | $V_{max}$ |
|---|---|---|
| Saline control | 4.0 ± 1.2 | 110 ± 9 |
| Naïve control | 4.0 ± 0.2 | 110 ± 11 |
| Desmethylimipramine | 4.0 ± 1.0 | 116 ± 9 |
| Tranylcypromine | 4.3 ± 0.8 | 121 ± 9 |
| Zimelidine | 4.6 ± 1.1 | 115 ± 11 |

Values are means ± SE for 4 determinations. $V_{max}$ is the maximal % increase compared to control values (no GABA). $EC_{50}$ (μM) is the concentration of GABA producing half maximal stimulation.

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# Effect of allopurinol on the first-pass metabolism of 6-mercaptopurine in the rat

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In a previous report [1], we investigated the characteristics of intestinal absorption of three anticancer antimetabolites, 5-fluorouracil, ftorafur and 6-mercaptopurine, by *in vitro* and *in situ* absorption techniques in rats. Most of the 6-mercaptopurine that was lost from the lumen appeared as its metabolite, 6-thiouric acid, in the lumen. A small serosal transfer of 6-mercaptopurine was confirmed using an *in vitro* method. These results indicate that the rate of intestinal first-pass metabolism of 6-mercaptopurine will be high after oral administration. Loo *et al.* [2] reported that the gastrointestinal absorption of 6-mercaptopurine is incomplete and variable in humans.

6-Mercaptopurine is normally metabolized to 6-thiouric acid by xanthine oxidase *in vivo* [3]. Allopurinol is used for the treatment of gout and other hyperuricemic states as a potent inhibitor of xanthine oxidase [4]. Elion *et al.* [5, 6] demonstrated that the catabolism of 6-mercaptopurine to 6-thiouric acid is diminished by the administration of allopurinol. This metabolic inhibition resulted in a several-fold potentiation of the antitumor activities of 6-mercaptopurine when tested against Adenocarcinoma 755 in mice. Coffey *et al.* [7] reported that daily administration of allopurinol orally to cancer patients almost completely inhibits the production of 6-thiouric acid from 6-mercaptopurine administered intravenously. Nonetheless, no effect was observable on the pharmacokinetics of 6-mercaptopurine in these patients [7].

The present study was undertaken to examine the effect of allopurinol on the first-pass metabolism of 6-mercaptopurine in rats.

## *Materials and methods*

*Materials.* Allopurinol and 6-mercaptopurine were purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan). 6-Thiouric acid was prepared according to the method reported by Elion *et al.* [8]. All other chemicals were of reagent grade.

*Animal experiments.* Male Wistar albino rats weighing 250–300 g were used throughout the study. The animals were housed in an air-conditioned room and maintained on a standard laboratory diet. The rats were fasted overnight but had free access to water. Animals were anesthetized with pentobarbital, given intraperitoneally. After complete anesthesia, 6-mercaptopurine and allopurinol dissolved in 0.1 M sodium hydroxide were administered simultaneously, orally by gastric intubation and intravenously into a femoral vein. Blood samples were removed periodically from the femoral vein. The area under the blood concentration versus the time curve (AUC) was calculated by the trapezoidal rule up to the last measurement.

*Analytical methods.* 6-Mercaptopurine in blood was analyzed by spectrofluorometry after modifying the method described by Maddocks [9]. A Shimadzu RF-510 spectrofluorometer (Shimadzu Co. Ltd., Kyoto, Japan) was used. Blood samples were diluted with 0.4 M NaOH and washed with ether containing 1.5% isoamyl alcohol. 6-Mercaptopurine in the aqueous layer was reacted with 0.3% phenylmercury acetate to produce 6-(phenylmercury)-mercaptopurine, which was extracted with toluene and then re-extracted with 0.1 M HCl. 6-Mercaptopurine in the acidic layer was oxidized by 2 mM potassium chromate to get fluorescence. The reaction was stopped by 0.4% sodium metabisulfite. After adding 5.0 M NaOH, relative fluorescence of the solutions was measured at 398 nm with an excitation wavelength of 288 nm.

## *Results and discussion*

Figure 1 shows the blood concentration of 6-mercaptopurine following intravenous administration. 6-Mercaptopurine was eliminated rapidly from the blood. Following oral or intravenous coadministration of allopurinol, the blood level of 6-mercaptopurine increased. Intravenous injection of allopurinol was more effective than oral administration. Allopurinol administered intra-