In the rat cerebral cortex, the benzoquinolizines and yohimbine interacted with [^{3}H]rauwolscine and [^{3}H]prazosin yielding displacement curves consistent with simple monomolecular interactions with labelled sites. This was also the case for RX 781094 at the α_1-site but not at the α_2-site where a slope of 0.79 was obtained. Slope values of less than unity have been associated with agonist interactions at [^{3}H]yohimbine or [^{3}H]rauwolscine-labelled α_2-sites in brain and platelet [9]. This probably relates to the association of agonists with guanine-nucleotide-sensitive high and low affinity sites.

In this context it is of interest to note that agonist activity has been reported with RX 781094 in the conscious rabbit and in isolated rabbit aorta [10] though the adrenoceptors in the rabbit aorta appear to be of the α_1-type.

It has previously been observed [8] using receptor binding methods that some α-adrenoceptor blockers have significantly greater affinities for the α_2-site in human platelet membranes than in rat cerebral cortex, and this has been taken as evidence that the α_2-adrenoceptors in these two tissues are not identical. In this study both yohimbine and Wy 26392 had a greater affinity for the platelet α_2-adrenoceptor than for the α_2-adrenoceptor in rat cerebral cortex, the difference being most marked with yohimbine. Thus, in the rat cerebral cortex yohimbine had the lowest affinity of this group of antagonists at the α_2-site but in the human blood platelet preparation yohimbine had the highest affinity of the group (Table 1). This again may indicate differences between α_2-adrenoceptors in these tissues.

Of the compounds tested, only RX 781094 showed significant differences in affinity between rat cerebral cortex and rat kidney cortex. The interpretation of this observation is, however, complicated by the possible agonist activity of this compound and subsequently its heterogeneous binding at least to cerebral cortical membranes.

In interpreting apparent differences in affinity of antagonists at α_2-adrenoceptors in these tissue preparations, it must be borne in mind that the physical properties of these preparations may influence access of the ligands to the receptor. These properties may differ between the tissues studied here. Problems of this sort may in future be clarified by work on solubilized receptor preparations.

In conclusion, these receptor-binding studies support the view that this group of substituted benzoquinolizines are potent and selective displacers of the α_2-adrenoceptor ligand [^{3}H]rauwolscine in rat cerebral cortex membranes, and in this tissue have a greater α_2:α_1 adrenoceptor selectivity than yohimbine. These benzoquinolizines have a similar affinity for α_2-adrenoceptors in both rat cerebral cortex and rat kidney cortex. The observation that yohimbine had the lowest affinity of this group of compounds at the α_2-adrenoceptor of rat cerebral cortex but the greatest affinity in human platelets provides further evidence of possible tissue or species differences in the α_2-adrenoceptor subtype.

In summary, a study using receptor-binding techniques with membrane preparations from the rat cerebral cortex, rat kidney cortex and human blood platelets showed that a series of substituted benzoquinolizines had a high affinity for α_2-adrenoceptors. In the rat cerebral cortex where α_1-binding was also studied, the benzoquinolizines had a much lower affinity for this site than for the α_2-site, their α_2:α_1 selectivity being substantially greater than that of yohimbine.

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REFERENCES

1. K. Starke, *Rev. Physiol. Biochem. Pharmac.* **88**, 199 (1981).
2. N. Lattimer, K. F. Rhodes, T. J. Ward, J. F. Waterfall and J. F. White, *Br. J. Pharmac.* **75**, 154P (1982).
3. R. P. McAdams and K. F. Rhodes, *Br. J. Pharmac.* **79**, 308P (1983).
4. K. Starke, E. Borowski and T. Endo, *Eur. J. Pharmac.* **34**, 385 (1975).
5. C. B. Chapleo, J. C. Doxey, P. L. Myers and A. G. Roach, *Br. J. Pharmac.* **74**, 842P (1981).
6. P. Greengrass and R. Bremner, *Eur. J. Pharmac.* **55**, 323 (1979).
7. T. Tanaka, R. Weitzell and K. Starke, *Eur. J. Pharmac.* **52**, 239 (1978).
8. Y. Cheung, D. B. Barnett and S. R. Nahorski, *Eur. J. Pharmac.* **84**, 79 (1982).
9. L. E. Limbird, *Trends Pharmac. Sci.* **4**, 135 (1983).
10. H. W. Dalrymple, C. A. Hamilton, J. A. M. Hannah and J. L. Reid, *Br. J. Pharmac.* **78**, 128P (1983).

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Binding of benzodiazepines to blood platelets from various species

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Specific high affinity binding sites for benzodiazepines have been firmly established in the mammalian brain [1–4]. Comparison of receptor affinities and pharmacological effects has suggested that these receptors probably mediate the anxiolytic, muscle-relaxant and anticonvulsant properties of these compounds [2, 4–6].

Benzodiazepine binding sites have also been demonstrated in peripheral tissues such as the heart [7], kidney [8, 9], lung [9], liver [9], ileal skeletal muscle [7], peritoneal mast cells [10] and platelets [11]. The peripheral-type binding site shows a different pharmacological profile to that of the established CNS sites [8, 9, 12, 13], in that whereas the affinity of diazepam and flunitrazepam for the peripheral-type binding site is only slightly lower than that for the CNS sites clonazepam, which has a high affinity for CNS sites, has a very low affinity for peripheral sites. The benzodiazepine Ro 5-4864 has a high affinity for peripheral sites [8, 12, 13] but a very low affinity for CNS sites [1, 3, 14, 15]. Recently, specific high affinity binding sites for [^{3}H] Ro 5-4864 have also been found in the brain [16, 17] but are distinct in both regional and subcellular distribution from the established CNS sites.

Increased diazepam binding has been demonstrated to renal membranes during deoxycorticosterone/salt hyper-

tension in rats [18] and to platelets and renal membranes from spontaneously hypertensive rats [19] suggesting that platelet (or peripheral) benzodiazepine binding sites may be involved in hypertension and may be useful as a marker for predisposition to this disorder. Monitoring of platelet benzodiazepine binding sites may also be useful in other disorders, especially those such as depression, anxiety and epilepsy, in which benzodiazepines find extensive use.

This study describes the measurement of [^{3}H]benzodiazepine binding to platelets from several species. [^{3}H]-Benzodiazepine binding to platelets could only be demonstrated in rodents, whereas in other mammalian species including man no binding could be demonstrated.

Materials and methods

Chemicals. [^{3}H]Flunitrazepam (sp. act. 78 Ci/mmole) was from Amersham International (Amersham, Bucks., U.K.) and was diluted (1:4) with unlabelled flunitrazepam immediately before use. [^{3}H]-Ro 5-4864 (sp. act. 73 Ci/mmole) was from NEN (Dreieich, F.R.G.) and was diluted (1:1) with unlabelled Ro 5-4864 before use. Flunitrazepam and Ro5-4864 were gifts from Roche Products Ltd. (U.K.). Prostaglandin E_1* (PGE_1; stored at 3 mM in 50% ethanol–H_2O at −20°) and 5-hydroxytryptamine-creatinine sulphate (5-HT) were from Sigma Chemical Co. (Poole, Dorset, U.K.). Adenosine diphosphate di-sodium salt (ADP) was from Boehringer Corporation (Dublin, Ireland).

Animals. Adult male Wistar rats, mice (LACA) and guinea pigs were used. Sheep, ox and pig were all slaughtered immediately before blood collection. Human blood samples were from healthy volunteers.

Collection of blood. Rats, mice and guinea-pigs were anaesthetized with halothane and blood was collected using a hypodermic syringe into sodium citrate (3.8% w/v; 1:9 v/v anticoagulant : blood) from dorsal aorta (rat) or by cardiac puncture (mice and guinea pig). For humans venous blood (18 ml) was collected into anticoagulant (as above) using a hypodermic syringe. For ox, sheep and pig, blood was collected from the cut jugular artery of a freshly-slaughtered animal and immediately mixed with citrate anticoagulant (1:9, v/v as above). After thorough mixing PGE_1 (3 μM) was added to the blood.

Preparation of platelet-rich plasma. Mixed citrated blood was centrifuged at room temperature at 270 *g* for 20 min. Platelet-rich plasma (PRP) was collected into a plastic tube using a plastic Pasteur pipette and then stored at room temperature before use. PGE_1 when used (3 μM) was added to citrated blood before centrifugation.

Preparation of platelet-free plasma. Platelet-rich plasma was centrifuged at 1200 *g* for 10 min before removal of platelet-free plasma (PFP) using a Pasteur pipette.

Preparation of washed platelets. PRP was centrifuged at 1200 *g* for 10 min before removal of PFP using a plastic Pasteur pipette. The platelet pellet was resuspended to the original volume of PRP by gentle agitation at room temperature in 15 mM Tris–citrate buffer, pH 7.4, containing 125 mM NaCl, 4 mM EDTA, 5 mM KCl, 5.5 mM glucose and 0.5 mM magnesium acetate (assay buffer).

Platelet counting. Platelet counting was performed using a Hawksley counting chamber under phase-contrast microscopy.

Assay for platelet binding of [^{3}H]*flunitrazepam and* [^{3}H]*Ro* 5-4864. Platelets (5×10^7 or 1×10^8 cells) were incubated for 30 min at 4° with assay buffer in a total volume of 1 ml in triplicate with [^{3}H]flunitrazepam (10 nM) or Ro 5-4864 (8 nM) in the absence or presence of 10 μM unlabelled flunitrazepam or Ro 5-4864, respectively. PGE_1 (3 μM), ADP (10 μM), ketanserin (1.5 μM) and 5-HT (1.5 μM) when used were preincubated with platelets at room temperature for 10 min before additions of flunitrazepam. When used PRP or PFP fractions were incubated with platelets, the volumes of these fractions were such as to be equivalent to that volume of plasma from which platelets were isolated.

After incubation of platelets with [^{3}H]benzodiazepine, the samples were rapidly filtered through Whatman glass-fibre filters (GF/B, 2.5 cm) and rapidly washed three times with 5 ml of cold buffer (the time for filtration and washing was 10 sec). The filters were transferred to vials containing 10 ml Triton X-100–toluene–PPO scintillation cocktail (500:1000:4; ml:ml:g) and radioactivity was counted in a Packard 300 Scintillation Spectrometer at 50% efficiency. In each case total binding or non-specific binding was determined as the mean (±S.E.) of three replicates. Specific binding was determined by subtraction of the mean value for non-specific binding from the mean value for total binding. In all cases the non-specific binding was due to the filter blank and at 10 nM [^{3}H]flunitrazepam and 8 nM [^{3}H]-Ro 5-4864 were found to be 30 and 19 fmole, respectively, and one-tenth of this value was taken as the limit of detection for specific binding.

Results and discussion

Species distribution of platelet benzodiazepine binding. The specific binding of [^{3}H]flunitrazepam found for platelets from several species is shown in Table 1. Specific binding was found for rat, mouse and guinea-pig platelets and for rat the binding was consistent quantitatively with that previously reported [11]. Ro 5-4864 at 1 nM final concentration reduced specific [^{3}H]flunitrazepam binding to rat, guinea pig and mouse platelets by 91, 51 and 68%, respectively, demonstrating that [^{3}H]flunitrazepam bound at a peripheral-type benzodiazepine binding site. However, no specific binding was detectable to platelets from human (both male and female), ox, pig and sheep. In all cases, platelets were prepared in the presence of 4 mM EDTA and 5 μM PGE_1 to prevent aggregation and to minimize shape change, respectively [20]. No specific binding of [^{3}H]-Ro 5-4864 (8 nM) could be detected to human platelets. In all cases total binding, non-specific binding and filter blank were equal within a value of one-tenth filter blank value. Thus it would seem unlikely that the inability to detect specific binding was due to the low affinity for flunitrazepam for peripheral-type sites on platelets from non-rodent species.

Effect of shape change on [^{3}H]*flunitrazepam binding.* In order to determine if the flunitrazepam binding to platelets was influenced by membrane changes induced as a result of platelet-shape change, the effect of such shape changes induced by ADP and 5-HT and inhibited by PGE_1 and ketanserin was investigated (Table 2). 5-HT- and ADP-induced shape changes increased [^{3}H]flunitrazepam binding to rat platelets whereas ketanserin and PGE_1-inhibition of shape change appeared to decrease [^{3}H]flunitrazepam binding. However, these effects were small and it is apparent that the absence or presence of [^{3}H]flunitrazepam binding was not directly related to platelet shape being either discoid or "spiny-sphered". Human platelet-rich plasma was prepared in the absence and presence of PGE_1 and [^{3}H]flunitrazepam binding measured in the absence and presence of PGE_1. In no case could specific [^{3}H]flunitrazepam binding be detected. Thus it seems improbable that the lack of detectable binding in human platelets was due to the state of platelet activation.

Absence of inhibitors or cofactors of [^{3}H]*flunitrazepam binding.* It was possible that a cofactor for [^{3}H]flunitrazepam binding was present in rat plasma or that an inhibitor of binding was present in plasma of species which showed no detectable binding. Rat platelets (both from platelet-rich plasma and washed platelets) were assayed for [^{3}H]-flunitrazepam binding in the presence of human

* Abbreviations: PGE_1, prostaglandin E_1; 5-HT, 5-hydroxytryptamine; PRP, platelet-rich plasma; PFP, platelet-free plasma.

platelet-free plasma, human platelet-rich plasma and rat platelet-free plasma. No appreciable inhibition of rat platelet [^{3}H]-flunitrazepam binding was found (Table 3) in the presence of human platelet-free plasma. Human platelets were assayed for [^{3}H]flunitrazepam binding in the presence of rat platelet-rich or platelet-free plasma. No specific binding of [^{3}H]flunitrazepam to human platelets could be detected in the presence of rat platelet-poor plasma. However, the expected specific binding of [^{3}H]flunitrazepam to rat platelets was found in the presence of human platelet-rich plasma.

Thus it seems unlikely that there existed in human plasma an inhibitor of [^{3}H]flunitrazepam binding to rat platelets or that there existed in rat plasma an activator of [^{3}H]-flunitrazepam binding to human platelets. It is, however, possible that a putative activator or inhibitor may not activate or inhibit binding to platelets from a different species.

In summary, although [^{3}H]flunitrazepam binding and Ro 5-4864 binding to peripheral-type sites on rat platelets could be detected as reported previously [11], and also to mouse and guinea-pig platelets, no specific binding of [^{3}H]flunitrazepam or [^{3}H] Ro 5-4864 could be detected to platelets from humans and several other species. The lack of detectable binding did not appear to be as a result of platelet shape change or due to the presence of an inhibitor in those species without detectable binding, or a cofactor in those species which demonstrated platelet benzodiazepine binding. The use of rat platelets as a model system for the study of the role of platelet peripheral-type benzodiazepine binding sites in human disorders should thus be treated with caution.

Table 1. Species distribution of platelet [^{3}H]flunitrazepam binding

Species	Specific binding (fmole/10^8 platelets ± S.E.)
Rat	106 ± 12 (5)
Mouse	583 ± 96 (3)
Guinea-pig	73 ± 10 (2)
Human	
male	n.d. (5)
female	n.d. (4)
Ox	n.d. (4)
Pig	n.d. (6)
Sheep	n.d. (4)

Standard errors are based on standard error of the mean of (n) experiments. In all cases where specific binding was not detectable (n.d.), non-specific binding and total binding were never greater than the filter blanks by more than 10%.

Table 2. Effect of shape change on rat platelet [^{3}H]flunitrazepam binding

	Specific binding (fmole/10^8 platelets ± S.E.)
Control	94 ± 4 (4)
+ ADP	108 ± 8 (4)
+ 5-HT	104 ± 8 (4)
+ PGE_1	74 ± 6 (2)
+ Ketanserin	84 ± 6 (4)

Results are expressed as mean ± standard error or mean of (n) experiments.

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REFERENCES

1. R. F. Squires and C. Braestrup, *Nature, Lond.* **266**, 732 (1977).
2. H. Möhler and T. Okada, *Science* **198**, 849 (1977).
3. R. C. Speth, G. W. Wastek, P. C. Johnson and H. I. Yamamura, *Life Sci.* **22**, 859 (1978).
4. J. F. Tallman, S. M. Paul, P. Skolnick and D. W. Gallagher, *Science* **207**, 274 (1980).
5. C. Braestrup and R. F. Squires, *Eur. J. Pharmac.* **48**, 263 (1978).
6. R. C. Speth, R. W. Johnson, J. W. Regan, T. D. Reisine, R. M. Kobayashi, N. Bresolin, W. R. Roeske and H. I. Yamamura, *Fedn Proc.* **39**, 3032 (1980).
7. W. R. Roeske and H. I. Yamamura, *Clin. Res.* **30**, 18A (1982).
8. J. W. Regan, H. I. Yamamura, S. Yamada and W. R. Roeske, *Life Sci.* **28**, 991 (1981).
9. C. Braestrup and R. F. Squires, *Proc. natn Acad. Sci. U.S.A.* **74**, 3805 (1977).
10. T. Taniguchi, J. K. T. Wang and S. Spector, *Life Sci.* **27**, 171 (1980).
11. J. K. T. Wang, T. Taniguchi and S. Spector, *Life Sci.* **27**, 1881 (1980).

Table 3. Assay for potential activators or inhibitors of human platelet [^{3}H]-flunitrazepam binding

	Specific binding (fmole/5 × 10^7 platelets ± S.E.)
Human PRP	n.d.
Human PRP + Rat PFP	n.d.
Human PRP + Rat PRP	120 ± 5
Rat PRP	107 ± 5
Rat PRP + Rat PFP	97 ± 6
Rat PRP + Human PFP	78 ± 2
Rat Platelets	122 ± 4
Rat PRP + Human PFP	87 ± 11
Rat PRP + Rat PFP	95 ± 13
Rat PRP + Human PRP	91 ± 3

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12. C. Braestrup and M. Nielsen, *Trends Neurosci.* **3**, 301 (1980).
13. J. W. Regan, W. R. Roeske, J. B. Malick, S. H. Yamamura and H. I. Yamamura, *Molec. Pharmac.* **20**, 477 (1981).
14. K. D. McCarthy and T. K. Harden, *J. Pharmac. exp. Ther.* **216**, 183 (1981).
15. C. Braestrup, E. Alberechtsen and R. F. Squires, *Nature, Lond.* **269**, 702 (1977).
16. H. Schoemaker, R. G. Boles, W. D. Horst and H. I. Yamamura, *J. Pharmac. exp. Ther.* **225**, 61 (1983).
17. J. G. Richards, H. Möhler and W. Haefely, *Trends Pharmac. Sci.* **3**, 233 (1982).
18. J. W. Regan, H. I. Yamamura, S. Yamada and W. R. Roeske, *Eur. J. Pharmac.* **67**, 167 (1980).
19. T. Taniguichi, J. K. T. Wang and S. Spector, *Eur. J. Pharmac.* **70**, 587 (1981).
20. G. V. R. Born, *J. Physiol., Lond.* **209**, 487 (1970).

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The effects of oral ginseng administration on the activities and isoenzyme profiles of murine lactate dehydrogenases

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The rhizome of the perennial herb *Panax ginseng* C. A. Meyer has been of importance in the practice of Chinese medicine for the past 2000 years [1], with a wide variety of curative properties ascribed to its use [2]. The active agents in the root extract are ginsenosides, which on acid hydrolysis yield the tetracyclic dammarene triterpenoids, 20*S*-protopanaxadiol and 20*S*-protopanaxatriol, and the pentacyclictriterpene, oleanolic acid [3, 4]. A number of physiological effects including the prevention of body temperature fluctuation following exposure to heat and cold stress [2], improved exercise rates and prolonged swimming times [5] and a reduction in the effects of X-ray irradiation [6] have been claimed for ginseng in laboratory animals. In biochemical terms, the intraperitoneal injection of ginsenosides into rats and mice has been reported to increase the rates of hepatic RNA and protein synthesis [7, 8] and to accelerate hepatic lipogenesis with accompanying decreases in liver glycogen, blood glucose and serum triacylglycerol levels [9]. Ginseng injection was also reported to increase the activity of the glycolytic enzyme pyruvate kinase (EC 2.7.1.40) [10] but to result in a loss of serine dehydratase activity (EC 4.2.21.13) [11].

Following the addition of ginseng to the culture medium of human diploid fibroblasts, significant increases were observed in the activities of phosphohexose isomerase (EC 5.3.1.9) and lactate dehydrogenase (EC 1.1.1.27), the principal gain being in the lactate dehydrogenase (LDH) isoenzyme fractions 2–5 [12]. The aim of the present study was to determine whether similar effects on LDH activity occurred *in vivo* in mice following the oral administration of ginseng.

Materials and methods. Two groups of 25 male mice, strain LACa, each weighing 26–32 g, were maintained on a 12 hr light, 12 hr dark regime, five to a group. Ginseng saponins in the form of a freeze-dried, aqueous extract supplied by Pharmaton S.A. (Lugano, Switzerland) were dissolved in water and administered to the test group at a rate of 8 mg/kg body wt, equivalent to approximately 40 mg of whole root/kg body wt per day, for a period of 30 days. After 21 days of treatment, the pharmacological activity of the ginseng saponins was verified by open-field stress tests [13]. The mice were killed by cervical dislocation, and the brain, heart, liver, skeletal muscle and testes immediately removed from each animal and stored at −20°. For the enzyme assays, tissues were homogenized in 0.01 M phosphate buffer, pH 7.4, to give an approximate concentration of 10% (w/v). The crude homogenate was centrifuged at 700 *g* for 10 min and the lactate dehydrogenase (LDH; EC 1.1.1.27) activity of each tissue was immediately determined in triplicate on the supernatant by the NADH/NAD-linked method of Wroblewski and LaDu [14], in which 1 unit of LDH activity is defined as a decrease in extinction of 0.001 per min, measured at 340 nm, pH 7.4 and 25°. The total protein of each preparation was assayed by the method of Hartree [15]. Isoenzymes were separated by polyacrylamide gel electrophoresis (PAGE) on rod gels of 7% acrylamide prepared by a modification of the Davis method [16]. Samples equivalent to 25 μg protein were run in a 12 hole rod gel electrophoresis chamber at 18–20° and a constant current of 3 mA per tube for approximately 2 hr, the exact time being determined by the rate of movement of bromophenol blue through the gels. Final visualization was by precipitation of reduced nitro-BT formazans [17]. The extinction of the individual isoenzyme bands was measured on a Cary 210 scanning densitometer (Varian Associates) at 540 nm and the activity of each isoenzyme calculated as a proportion of the relevant total activity. The total LDH activity and the activities of each of the isoenzymes of the five tissues tested from the ginseng-treated and the control animals were statistically compared by the non-parametric two-tailed Mann–Whitney U-test [18].

Results and discussion. Following ginseng administration, LDH specific activity was only increased significantly in the hepatic homogenates (Table 1). There were small but non-significant increases in the activity of LDH from the heart and testis samples of ginseng-treated animals; the LDH activity of the brain samples showed no change while in the skeletal muscle preparations LDH exhibited a small but non-significant decrease in activity (Fig. 1). The administration of the ginseng saponins had no significant effect on the LDH isoenzyme profiles of any of the five tissues tested (Table 2).

In rats 20–25% of a dose of ginsenosides administered via the drinking water was absorbed [19] and was measurable in the heart, lung, liver, kidney and spleen within 1.0 hr, the maximum levels being attained after 1.5 hr [20]. Only the brain failed to show any trace of the ginsenosides, suggesting an inability to cross the blood–brain barrier. Thus with the exception of the brain, the observed tissue-specific differences in LDH activity in the mouse following oral ginseng administration can be considered real rather than representing a failure of the active principle(s) in the extract to reach their particular, potential site of action.

Lactate dehydrogenase is a tetrameric enzyme, its pattern of activity in specific tissues being determined by varying combinations of the A and B structural sub-units [21]. In the mouse these are coded for respectively by the Ldh-1 locus on chromosome 7 and Ldh-2 locus, as yet