total number of binding sites (Bmax) was 52 ± 17 fmoles × mg protein^{-1}, and the affinity of [^{3}H]-DHA for the splenic binding sites was 0.69 ± 0.17 nM. Pharmacological characterization of the labelled sites demonstrated that the binding of [^{3}H]-DHA could be displaced by a number of β-adrenergic agonists and antagonists with relative affinities that match their pharmacological potency at the β-adrenoceptor.

However, whereas (−)-timolol, a non-selective β-adrenoceptor antagonist, competed for a single population of high affinity sites ($K_i = 0.69$ nM), agents that demonstrate high selectivity for β_1 or β_2 adrenoceptors ((±)-practolol, (±)-atenolol (β_1), or procaterol (β_2)) produced flattened displacement curves. Analysis of the competition curves suggests that 30–35% of the labelled sites have high affinity for β_1 drugs, whereas the remainder can be displaced with high affinity by β_2 agents.

In an attempt to determine whether β_1 or β_2 receptors were associated with presynaptic sites, rats were treated with 6-hydroxydopamine (2 × 50 mg/kg + 2 × 70 mg/kg i.v.) over two weeks to destroy noradrenergic nerve terminals in the spleen. This treatment produced a severe depletion (control 1130 ± 84, treated 271 ± 31 pmole × gm^{-1}) of splenic noradrenaline that was maintained for at least seven days after the last injection. However, membranes prepared from treated rats possessed β-adrenoceptor binding sites with properties (number of sites, affinity for [^{3}H]-DHA, proportions of β_1 and β_2 receptor sites) that were virtually identical to controls.

Although it was not possible to identify presynaptic β-adrenoceptors in rat spleen, it appears possible that these sites represent a very small proportion of the total β-adrenoceptor population. However, the present experiments do suggest that since the properties of [^{3}H]-DHA binding sites were unaltered when presynaptic nerve terminals were destroyed by 6-hydroxydopamine, both β_1 and β_2 adrenoceptor subtype co-exist postsynaptically in this tissue.

References

BARNETT, D.B., RUGG, E.L. & NAHORSKI, S.R. (1978). Direct evidence of two types of β-adrenoceptor binding site in lung tissue. *Nature, Lond.* **273**, 166–168.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.* **60**, 481–497.

NAHORSKI, S.R. (1978). Heterogeneity of cerebral β-adrenoceptor binding sites in different vertebrate species. *Eur. J. Pharmac.* **51**, 199–209.

WOLFE, B.B., HARDEN, K.T. & MOLINOFF, P.B. (1977). *In vitro* study of β-adrenergic receptors. A. Rev. Pharmac. Toxicol. **17**, 575–604.

Effect of diazepam and its metabolites on the binding of L-tryptophan to human serum albumen

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Benzodiazepines compete with L-tryptophan for the binding site on the albumen molecule. In the rate, chlordiazepoxide (20 mg/kg i.p.) produces an increase in free tryptophan in plasma with an increase in the concentration of tryptophan and 5-hydroxytryptamine in the brain (Bourgoin, Héry, Ternaux & Hamon, 1975). We have studied the effects of diazepam and its metabolites on the affinity constant of L-tryptophan for purified human serum albumen (HSA), and the effect of diazepam on tryptophan binding in normal human serum using a drug concentration (35 μM) which is somewhat higher than that which would be expected in the therapeutic situation (Bond, Hailey & Lader, 1977).

In the studies with purified HSA, solutions were prepared containing L-([^{14}C]-methylene) tryptophan (0.17 μCi/ml:3 μM), L-tryptophan (six concentrations between 12 and 200 μM), HSA (approximately 75 μM, Sigma – essentially fatty acid free), PEG 200 (0.5%) and its benzodiazepine (50 μM) where appropriate, in tris buffer (50 μM: 7.4). These were left for 1 h at room temperature, and the free L-tryptophan separated by ultrafiltration through Amicon CF 25 cones (5 min at 900 g). Radioactivity in the mixture and ultrafiltrate was measured by liquid scintillation counting. Total L-tryptophan concentrations were determined using an amino acid analyser, and the albumen concentration by the bromo-cresol green assay. In the studies with human serum, blood was taken at 0900 h from five healthy male subjects. Portions of the pooled serum were prepared containing L-([^{14}C]-methylene) tryptophan (0.033 μCi/ml:0.6 μM), and diazepam (35 μM) or saline (control), and pH was maintained under an atmosphere of 95% air and 5% carbon dioxide. The free and total tryptophan concentrations were determined as before.

Linear regressions were fitted to the data for the Scatchard plots ($P<0.01$) and gave intercepts on the abscissae close to unity for control and all drugs (0.93–1.11). The mean association constant of L-tryptophan for HSA (17.7 × 10^{3}M^{-1}) was changed by diazepam ($K = 8.6 \times 10^3 M^{-1}$: $P<0.01$), temazepam ($K = 8.8 \times 10^3 M^{-1}$: $P<0.05$), oxazepam ($K = 9.4 \times 10^3 M^{-1}$: $P<0.05$), and nordiazepam ($K = 11.9 \times 10^3 M^{-1}$: $P<0.01$), but there were no differences between the association constants of in-

dividual drugs. The mean percentage of free tryptophan in serum (28.1% ± 1.7) was not changed with diazepam (27.6% ± 1.1).

It is evident that the effect of diazepam and its metabolites on the interaction between L-tryptophan and albumen is competitive, and involves a single binding site. However, serum concentrations of diazepam and its metabolites in man are too low to produce an increase in free tryptophan by displacement from albumen.

References

BOND, J.B., HAILEY, D.M. & LADER, M.H. (1977). Plasma concentrations of benzodiazepines. *Br. J. clin. Pharmac.*, **4**, 51–56.

BOURGOIN, S., HERY, F., TERNAUX, J.P. & HAMON, M. (1975). Effects of benzodiazepines on the binding of tryptophan in serum. Consequences on 5-hydroxyindoles concentration in the rat brain. *Psychopharmacologia Communications*, **1**, 209–216.

The binding properties of [^{3}H]-mepyramine in the brain of the guinea-pig and the rat

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[^{3}H]-Mepyramine binds selectively to histamine H_1 receptors in homogenates of longitudinal muscle from guinea-pig ileum (Hill, Young & Marrian, 1977) and has been used to demonstrate the presence of H_1 receptors in the brain of the guinea-pig (Hill & Young, 1978) and the rat (Chang, Tran & Snyder, 1978). However, the regional distribution of H_1 receptors in guinea-pig brain (Hill, Emson & Young, 1978) differs from that in the rat (Chang *et al.*, 1978), the most striking contrast being in the large amount of receptor material in guinea-pig cerebellum. Our own unpublished measurements on the rat have confirmed this difference, but also appeared to suggest that in certain respects the properties of [^{3}H]-mepyramine binding in rat brain did not correspond well with those in guinea-pig brain.

The binding of [^{3}H]-mepyramine to H_1 receptors in a membrane fraction from guinea-pig brain, measured in Na-K phosphate buffer (50 mM pH 7.5), and defined as the binding sensitive to 2×10^{-6}M promethazine, appears to saturate with a K_d near 1.7 nM, in reasonable accord with the value expected from measurements on the inhibition by mepyramine of the contractile response to histamine of guinea-pig ileum. In contrast, the binding to an analogous fraction from rat brain did not obviously saturate below 32 nM and in each of 4 experiments analysis of the curve, using the method of Wilkinson (1961), indicated an apparent K_d for [^{3}H]-mepyramine in the region of 10 nM. The discrepancy was still apparent when the affinity of mepyramine for H_1 receptors in rat brain was determined from the inhibition of the binding of [^{3}H]-mepyramine (1 nM) by non-radioactive mepyramine, where the IC_{50} was 1.2×10^{-8}M (cf IC_{50} 1.8×10^{-9}M in guinea-pig brain under the same conditions. There was a marked difference in the potency of the isomers of chlorpheniramine, in agreement with Chang *et al.* (1978), but the potency of the (+)-isomer was again much greater in the guinea-pig (IC_{50} 1.8×10^{-9}M) than the rat (1.0×10^{-8}M). However, not all ligands showed differences of this magnitude. Promethazine appeared to be at least as potent, if not more so, in the rat as in the guinea-pig and the difference in the IC_{50} values for chlorpromazine was also small. Histamine was practically equipotent in the two species. It must be noted, however, that the accuracy of these measurements in the rat is limited by the relatively large proportion of non-specific binding (*c.* 70% with 1 nM [^{3}H]-mepyramine).

The differences between the species might reflect either an actual difference between the H_1 receptors or the presence of a relatively large proportion of secondary binding sites in the rat. Some support is given to the latter possibility by the presence of low-affinity promethazine-sensitive binding of [^{3}H]-mepyramine in rat intestinal smooth muscle, a tissue notably insensitive to histamine.

We thank the M.R.C. for financial support. S.J.H. holds a S.R.C. Research Studentship.

References

CHANG, R.S.L., TRAN, V.T. & SNYDER, S.H. (1978). Histamine H_1 receptors in brain labeled with ^{3}H-mepyramine. *Eur. J. Pharmac.*, **48**, 463–464.

HILL, S.J., EMSON, P.C. & YOUNG, J.M. (1978). The binding of [^{3}H]-mepyramine to histamine H_1 receptors in guinea-pig brain. *J. Neurochem.*, in press.

HILL, S.J. & YOUNG, J.M. (1978). Evidence for the presence of histamine H_1 receptors in guinea-pig brain. *Br. J. Pharmac.*, **63**, 394P–395P.

HILL, S.J., YOUNG, J.M. & MARRIAN, D.H. (1977). Specific binding of ^{3}H-mepyramine to histamine H_1 receptors in intestinal smooth muscle. *Nature, Lond.*, **260**, 163–165.

WILKINSON, G.N. (1961). Statistical estimations in enzyme kinetics. *Biochem. J.*, **80**, 324–332.