Pharmaceutical Research, Vol. 6, No. 5, 1989

Report

Kinetics of Drug Action in Disease States. XXXI. Effect of Experimental Hyperthyroidism on the Hypnotic Activity of a Benzodiazepine (Oxazepam) in Rats

Judith S. Walker,[1] Patricia M. Klockowski,[1] and Gerhard Levy[1,2]

Received August 4, 1988; accepted December 28, 1988

This investigation was designed to determine the effect of experimental hyperthyroidism on the hypnotic activity of a benzodiazepine and on the binding characteristics of the benzodiazepine receptor complex. Rats were made hyperthyroid by subcutaneous implantation of slow release pellets containing L-thyroxine. This treatment produced the characteristic symptoms of hyperthyroidism: increased serum thyroxine concentrations, increased heart weight and body temperature, and decreased serum protein concentrations. The hyperthyroid rats and a parallel group of normal animals (with drug-free pellets implanted subcutaneously) were slowly infused intravenously with oxazepam until they lost their righting reflex. The hyperthyroid rats required a significantly larger dose of the benzodiazepine and their total serum and cerebrospinal fluid concentrations of oxazepam (but not the serum concentration of free drug and the brain concentration) at the onset of loss of the righting reflex were modestly but statistically significantly lower than those of the normal rats. The receptor density and affinity for diazepam in hyperthyroid rats were not significantly different from those of the normal animals. Hyperthyroidism apparently affects the pharmacokinetics of oxazepam but has, at best, only a small effect on the pharmacodynamics (hypnotic activity) of the drug.

KEY WORDS: hyperthyroidism; oxazepam; rat; benzodiazepine; pharmacodynamics; receptors.

INTRODUCTION

The thyroid hormones thyroxine and triiodothyronine inhibit flunitrazepam binding to the benzodiazepine receptor complex *in vitro* (1). The effect is stereoselective. Interestingly, the endocrinologically inactive D-thyroxine is about 40-fold more effective as an inhibitor than the active L-isomer and more than 60 times as effective as triiodo-L-thyronine. Moreover, the average concentrations of the thyroid hormones in the brain are much lower than those needed to inhibit specific flunitrazepam binding *in vitro* (1). In a subsequent study, a 21% decrease in the number of flunitrazepam receptor binding sites, but no change in receptor affinity, was found in rats made hyperthyroid with L-triiodothyronine, whereas in thyroidectomized animals there was an increase in the number of binding sites, again without a change in affinity (2). However, despite the down-regulation of benzodiazepine binding sites in hyperthyroid rats, experimental hyperthyroidism had no effect on the convulsive dose of pentylenetetrazol or on the anticonvulsant activity of flurazepam (as reflected by the pentylenetetrazol seizure threshold dose) in rats (3). Thus, the binding data suggest a modulatory role of thyroid hormones on the benzodiazepine receptor complex (2), whereas the *in vivo* results indicate no alteration of rat brain benzodiazepine receptor function (3).

An *in vivo* animal model for pharmacodynamic studies of benzodiazepines has recently been developed in this laboratory (4) which permits assessment of drug concentration (rather than dose) versus effect relationships without interference by possible alterations of drug binding or disposition. This animal model, which is based on the determination of drug concentrations in the virtually protein-free cerebrospinal fluid at the onset of a defined pharmacologic end point, has been used to determine the effect of experimental hyperthyroidism on the hypnotic activity of the benzodiazepine drug oxazepam. This particular benzodiazepine was selected because, unlike others in its class, it is not metabolized to pharmacologically active metabolites. In addition, the effect of hyperthyroidism on the *in vitro* binding of diazepam to the benzodiazepine receptor complex was determined.

MATERIALS AND METHODS

Female, inbred Lewis rats (LEW/CrlBR, Charles River Breeding Laboratories, Wilmington, Mass.), weighing 180 to 220 g when received, were used in this investigation. They were maintained on a diet of Charles River RMH 1000 and housed in a temperature-, humidity-, and light-controlled room. Hyperthyroidism was produced by subcutaneous implantation of a pellet containing 15 mg L-thyroxine (Innova-

[1] Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, New York 14260.
[2] To whom correspondence should be addressed.

0724-8741/89/0500-0404$06.00/0

tive Research of America, Toledo, Ohio); control animals received a drug-free pellet. On the 27th day after pellet implantation, all rats had a cannula implanted in the right jugular vein under brief and light ether anesthesia. Food was withdrawn that evening but water was available to the animals until the next morning, the day of the pharmacodynamic study. Experiments were performed between 9 AM and 2 PM on that day.

Oxazepam (1 mg/ml of 33% polyethylene glycol 3350 in water) was infused intravenously at a rate of 0.412 mg/min, while the animals were on a heating pad, until the onset of loss of righting reflex which was determined without a nociceptive stimulus. Cerebrospinal fluid (CSF) from the cisterna magna, blood (for serum) from the abdominal aorta, and the brain were obtained immediately upon loss of the righting reflex and assayed for oxazepam by high-performance liquid chromatography (5). Serum protein binding was determined at 37°C by equilibrium dialysis (6).

For the *in vitro* benzodiazepine receptor binding study, rats were prepared as for the pharmacodynamic study except that they were not cannulated. On the 28th day after pellet implantation, the animals were decapitated between 9 AM and 10 AM under light ether anesthesia, the brain was removed, and the cerebral cortex was dissected rapidly on ice, frozen in a vial immersed in dry ice, and then stored at −80°C. Tritiated diazepam binding determinations were performed as previously described (6).

The statistical significance of differences between the control and the hyperthyroid groups was determined by Student's unpaired *t* test or by the Mann–Whitney test. An outlier in the receptor binding study was identified on the basis of a chi-square goodness-of-fit test (7).

RESULTS

The hyperthyroid rats used in the pharmacodynamic study and their normal controls are described in Table I. The hyperthyroid animals had a significantly higher body temperature, heart weight, and serum thyroxine concentration than the controls and a lower concentration of total protein in serum. Average body weights were comparable.

The results of the pharmacodynamic study are summarized in Table II. The hyperthyroid animals required a significantly larger dose of oxazepam to produce loss of the righting reflex. However, the concentrations of the drug in plasma and cerebrospinal fluid at the onset of the pharmacologic end point were statistically significantly, although quantitatively only modestly, lower than in the control rats. There were no significant differences between the hyperthyroid and the normal control animals with respect to oxazepam concentrations in serum water (i.e., free drug) and brain. Serum free fraction values of oxazepam exhibited substantial interindividual variability and a tendency (although not statistically significant) to increase in the hyperthyroid animals.

Hyperthyroidism in the thyroxine-treated animals used for the benzodiazepine receptor binding study was confirmed by the elevated serum thyroxine concentrations and heart weights (Table III). There was no apparent difference in benzodiazepine receptor complex binding capacity (B_{max}) and affinity (K_d) for diazepam between the hyperthyroid and the control rats (Table III).

DISCUSSION

Thyroid disorders can have pronounced effects on the pharmacokinetics of drugs (8), including oxazepam (9), but little is known about the pharmacodynamic implications of thyroid disease. The usual reports of drug action in thyroid disease relate pharmacologic response to dose, rather than concentration, so that the relative roles of pharmacokinetics and pharmacodynamics in altering drug action cannot be established.

The use of subcutaneously implanted, slow-release pellets of L-thyroxine was successful, under the conditions of this investigation, to produce hyperthyroidism as reflected by the typical physiologic symptom complex associated with this condition, i.e., increased heart weight (10), increased body temperature (11), and hypoproteinemia (9), in addition to the elevated serum thyroxine concentrations. A preceding study of the pharmacodynamics of diazepam and its metabolites (including oxazepam) in rats has shown that the cerebrospinal fluid is the optimum sampling site for determination of drug concentrations in relation to the hypnotic effect (4). Serum and brain concentrations are affected by changes in nonspecific protein binding and there is a substantial distribution disequilibrium of the benzodiazepines between the brain as a whole and sites of action within the brain (4). Pharmacodynamic and radioreceptor assay studies have shown no evidence of active oxazepam metabolites in rats (12).

In this investigation, oxazepam concentrations in the

Table I. Effect of Experimental Hyperthyroidism on Certain Physiologic and Biochemical Indices in Rats[a]

Characteristics	Controls	Hyperthyroid	Statistical Significance
No. of animals	14	12	
Body weight, g	204 ± 13	206 ± 14	NS[b]
Body temperature, °C	38.8 ± 0.5	39.8 ± 0.7	<0.001[b]
Heart weight, mg/100 g body wt	347 ± 23	496 ± 35	<0.001[b]
Serum thyroxine conc., μg/100 ml	4.15 ± 0.97	35.8 ± 11.0	<0.001[c]
Serum total protein conc., g/100 ml	6.09 ± 1.08	5.20 ± 0.45	<0.03[c]

[a] Results are reported as mean ± SD in all tables.
[b] Unpaired *t* test.
[c] Mann–Whitney *U* test.

Table II. Effect of Experimental Hyperthyroidism on the Concentrations of Oxazepam in Rats at Onset of Loss of the Righting Reflex[a]

Variable	Controls	Hyperthyroid	Statistical Significance
Infusion time, min	9.74 ± 1.15	11.1 ± 0.8	<0.005[b]
Total dose, mg/kg	19.7 ± 2.0	22.0 ± 1.9	<0.005[b]
Serum concentration, mg/liter			
Total drug	12.4 ± 1.8	10.9 ± 0.9	<0.05[c]
Free drug	1.17 ± 0.54	1.25 ± 0.50	NS[b]
Brain concentration, mg/kg	13.6 ± 2.3	12.9 ± 1.6	NS[b]
CSF concentration, mg/liter	1.19 ± 0.22 (11)	0.985 ± 0.225 (11)	<0.05[b]
Free fraction in serum × 100	9.44 ± 4.26	11.3 ± 4.2	NS[b]

[a] Number of animals as in Table 1 unless stated otherwise in parentheses.
[b] Unpaired *t* test.
[c] Mann–Whitney *U* test.

CSF at the onset of loss of the righting reflex were about 17% lower in hyperthyroid than in euthyroid (control) rats, a statistically significant difference. By itself, this suggests an increased central nervous system sensitivity to the depressant effect of oxazepam in hyperthyroidism. The increase in the effective dose of oxazepam in the hyperthyroid rats despite lower or unchanged drug concentrations at the onset of action suggests a change in the pharmacokinetics of the drug, consistent with the reported increase of oxazepam clearance in hyperthyroid patients (9).

In vitro receptor binding studies were performed with tritiated diazepam rather than with oxazepam. The latter benzodiazepine was not available to us in tritiated form with a specific activity high enough for receptor binding studies. Also, oxazepam is not as suitable as diazepam because of its lower affinity for benzodiazepine receptors (13–15). On the other hand, *in vivo* pharmacodynamic studies with diazepam are complicated by the formation of three active metabolites which together contribute substantially to the total pharmacologic effect (4). Diazepam, due to its higher receptor affinity, may also be less sensitive than oxazepam to possible disease-associated alterations in the concentration or activity of endogenous ligands that can compete for benzodiazepine receptor sites. This would be a lesser problem for *in vitro* receptor binding determinations because they include tissue washing and dilution procedures. Endogenous ligands of central benzodiazepine receptors are known to exist (16) and their concentration or activity can apparently change in some disease states (17).

The absence of significant alterations in the diazepam binding characteristics of the benzodiazepine receptors in hyperthyroid rats appears to be contrary to the pharmacodynamic results (i.e., the difference of oxazepam concentrations in CSF at onset of action) and to the reported *decrease* in the capacity (but not the affinity) of flunitrazepam receptor sites in hyperthyroid rats (2). However, both of these changes were small, 17 and 21%, respectively, and the coefficients of variation of our binding capacity (B_{max}) estimates were of a similar magnitude. This may also explain the report that "experimental hyperthyroidism does not seem to alter benzodiazepine/GABA function as revealed by seizure thresholds in mature rats" (3). Irrespective of these uncertainties it can be concluded that experimental hyperthyroidism has little or no effect on the central nervous system depressant activity of the benzodiazepine drug oxazepam in rats.

ACKNOWLEDGMENTS

This work was supported in part by Grant GM 20852 from the National Institute of General Medical Sciences, National Institutes of Health. Mr. David M. Soda provided competent technical assistance.

Table III. Effect of Experimental Hyperthyroidism on Benzodiazepine Receptor Characteristics in Rats[a]

Parameter	Controls	Hyperthyroid[b]	Statistical Significance
Serum thyroxine conc., μg/100 ml	6.08 ± 1.30	44.0 ± 9.8	<0.001[c]
Heart weight, mg/100 g body wt	297 ± 15	489 ± 34	<0.001[c]
B_{max}, pmol/mg protein	0.460 ± 0.108	0.556 ± 0.127	NS[d]
K_d, n*M*	2.86 ± 0.62	2.71 ± 0.56	NS[d]

[a] Parameters for specific binding of diazepam in nine rats per group.
[b] Excluding one outlier with B_{max} = 0.593 pmol/mg protein and K_d = 11.6 n*M*. This exclusion had no effect on the outcome of the statistical tests.
[c] Mann–Whitney *U* test.
[d] Unpaired *t* test.

REFERENCES

1. A. Nagy and A. Lajtha. *J. Neurochem.* 40:414–417 (1983).
2. J. H. Medina and E. De Robertis. *J. Neurochem.* 44:1340–1344 (1985).
3. C. K. Atterwill and D. J. Nutt. *J. Pharm. Pharmacol.* 35:767–768 (1983).
4. P. M. Klockowski and G. Levy. *J. Pharmacol. Exp. Ther.* 244:912–918 (1988).
5. P. M. Klockowski and G. Levy. *J. Chromatogr.* 422:334–339 (1987).
6. P. M. Klockowski and G. Levy. *J. Pharmacol. Exp. Ther.* 245:508–512 (1988).
7. J. H. Zar. *Biostatistical Analysis,* 2nd ed., Prentice–Hall, Englewood Cliffs, N.J., 1984, pp. 40–41.
8. P. O'Connor and J. Feely. *Clin. Pharmacokin.* 13:345–364 (1987).
9. A. K. Scott, A. S. M. Khir, P. D. Bewsher, and G. M. Hawksworth. *Br. J. Clin. Pharmacol.* 17:49–53 (1984).
10. A. J. Prange, Jr., M. A. Lipton, R. B. Shearin, and G. N. Love. *Biochem. Pharmacol.* 15:237–248 (1966).
11. R. D. Harbison and B. A. Becker. *J. Pharm. Sci.* 58:183–185 (1969).
12. J. Dingemanse, F. A. E. Sollie, D. D. Breimer, and M. Danhof. *J. Pharmacokin. Biopharm.* 16:203–228 (1988).
13. H. Mohler and T. Okada. *Science* 198:849–851 (1977).
14. P. A. Borea and A. Bonora. *Biochem. Pharmacol.* 32:603–607 (1983).
15. W. Sieghart and A. Schuster. *Biochem. Pharmacol.* 33:4033–4038 (1984).
16. E. DeRobertis, C. Pena, A. C. Paladini, and J. H. Medina. *Neurochem. Int.* 13:1–11 (1988).
17. K. D. Mullen, J. V. Martin, W. B. Mendelson, M. L. Bassett, and E. A. Jones. *Lancet* 1:457–459 (1988).