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Review

Benzodiazepines: Are They of Natural Origin?

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Three research groups have provided evidence that benzodiazepines might be also of natural origin. In the brain of different species including humans and in several plant products, desmethyldiazepam and diazepam are detectable by immunological methods and gas chromatography—mass spectrometry. Thus, benzodiazepines represent natural drugs which may be incorporated into animals and humans through plant products. Whether the measured low concentrations (ranging from 0.01 up to 600 ng/g wet weight) have any biological role or clinical significance remains to be determined.

KEY WORDS: benzodiazepines; animals; plants; human; natural drugs.

INTRODUCTION

Many drugs are of a natural plant origin (e.g., alkaloids). More recently it has been elucidated that morphine and codeine are present in different mammalian tissues (1) and the skeleton of morphine can be synthesized by the rat liver (2). Other examples of "natural" drugs which could be isolated from human or animal tissues are represented by *S*-(carboxymethyl)-cysteine (3) and *N*-acetyl-L-cysteine (4,5). Whereas both of the latter compounds can be regarded as simple metabolites of the essential amino acid cysteine, the complicated structure of the opioids requires a variety of metabolic steps in their biosynthesis.

Similarly, benzodiazepines represent drugs whose chemical structure is rather complex and which were discovered serendipitously in 1957 (6). Their pharmacological actions are mediated by a specific benzodiazpine– × $GABA_A$–chloride channel receptor complex whose molecular structure has recently been described (7). However, it is still unknown whether endogenous ligands exist for this receptor complex (8). Among the many candidates, a peptide with anxiogenic and diazepam-binding inhibitory properties, isolated from rat brain, could be one putative endogenous ligand (9).

Some parallelism in the biochemical research on opioids and benzodiazepines seems to exist. The response to both groups of drugs is accomplished by specific receptors in the brain and their action can be reversed by specific receptor antagonists. In the brain endogenous opiate peptides exist which bind to the central nervous system (CNS) opiate receptors. Because of the existence of receptors for benzodiazepines, it appears logical to search for endogenous ligands. Whether the parallelism between opioids and benzodiazepines can be extended to the above-mentioned mammalian biosynthesis of morphine and codeine (1,2) is speculative.

DETECTION OF DESMETHYLDIAZEPAM

The existence of benzodiazepine-like molecules in the brain was first verified by an antibenzodiazepine monoclonal antibody (10). This immunoreactivity was purified to homogeneity from bovine brain by immunoaffinity chromatography, gel filtration, and two reversed-phase high-performance liquid chromatographic (HPLC) steps. The substance was characterized as *N*-desmethyldiazepam (nordiazepam) by mass spectrometry, HPLC, spectrophotometry, benzodiazepine receptor binding, and immunological techniques. Benzodiazepine-like immunoreactivity was also found in six human brains that had been stored in paraffin since 1940, as well as in rat brains (11).

The yield of desmethyldiazepam from the richest bovine brain was 600 ng/g of wet cerebellum and cortex. However, the yield of activity from rat brain and cattle brain was lower by a factor of 100. These differences might reflect differential exposure of the animals to environmental benzodiazepines (11).

Similarly, desmethyldiazepam was purified from male rat brain by C-18 Sep-pak cartridge and reversed-phase HPLC. Defined HPLC fractions were analyzed by mass spectrometry. Amounts of about 3 ng/g were found in cortex, cerebellum, and hypophysis. In addition, lower amounts of desmethyldiazepam (0.3 ng/g) could also be detected in wheat grains (12).

Following different extraction and purification steps including reversed-phase HPLC and employing the stable isotope dilution technique (using deuterated desmethyldiazepam as internal standard) for quantification by gas chromatography/mass spectrometry, we could analyze desmethyldiazepam at much lower concentrations (range, 0.01 to 0.04 ng/g wet weight) in the brains of several species of different evolutionary stages, such as salmon, frog, monitor, rat, cat,

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dog, deer, bovine, chicken, hen, and human. In fresh, drug-free human blank plasma, maximal levels of 0.01 ng/ml were measured. In some plant products (e.g., brown lentils, yellow soy beans, unpealed rice, maize corn) similar minute amounts (0.003 to 0.015 ng/g) could be identified (13).

DETECTION OF DIAZEPAM

During the various purification steps for desmethyldiazepam, HPLC fractions with the retention time of diazepam were also analyzed for this benzodiazepine by gas chromatography/mass spectrometry. In rat cortex, cerebellum, hypophysis, and adrenals, amounts between 3 and 6 ng/g, and in wheat grains amounts of 0.2 ng/g, were estimated (12). Again, by employing stable isotope dilution techniques (using deuterated diazepam as internal standard) for quantification, we found low concentrations of diazepam in brains of salmon, frog, monitor, rat, cat, and dog (0.005 to 0.02 ng/g). Likewise, plant products, such as potato tuber, yellow soy beans, unpealed rice, and mushrooms, contained only traces between 0.002 and 0.05 ng/g (13).

DETECTION OF OTHER BENZODIAZEPINES

It has been suggested that oxazepam might also be present in some preparations from rat and bovine brain (11). In recent experiments we could identify oxazepam in low amounts (0.001 to 0.02 ng/g) in animal brain and liver (Unseld, Fischer, and Klotz, unpublished observations).

Most recently, traces of delorazepam, deschlorodiazepam, delormetazepam, lormetazepam, delorazepam and isodiazepam have been identified in wheat grains and potato tuber (17).

DISCUSSION

There is accumulating evidence that diazepam and its major biologically active metabolite desmethyldiazepam represent natural products. These intriguing findings are based mainly on sensitive and specific gas chromatography/mass spectrometry analysis. Measurements with biological methods, such as radioreceptor assay, confirm these observations (11–13). However, since such assays are characterized by a limited specificity due to cross-reacting benzodiazepine-like material, the results provided by mass spectrometry appear more reliable.

By comparing brain concentrations of desmethyldiazepam observed by three independent research groups, it is obvious that they differ considerably, e.g., ranges of 6 to 600 ng/g (11), 3 to 5 ng/g (12), and 0.01 to 0.04 ng/g (13) are reported. This discrepancy may be attributed to differences in methodology. Whereas the first two research groups did not use internal standards for quantification, we applied the stable isotope dilution technique for exact calculations (13). Since brain concentrations between 3 and 30 ng/g are obtained after therapeutic doses of benzodiazepines (14), "endogenous" brain levels in the range of 3 to 600 ng/g should result in pharmacological effects. Therefore it appears conceivable that our lower brain levels might represent a somewhat more realistic picture.

Diazepam and desmethyldiazepam were also detected in different plant products (12,13) which serve animals and humans as natural food. The origin and/or biosynthesis of both benzodiazepines is still unknown. It remains to be investigated whether animals are able to synthesize them or whether exogenous sources (e.g., microorganisms, plants) account for the presence of minute amounts in the body of different species. It is noteworthy that cyclopeptine, a benzodiazepine alkaloid (see Fig. 1), can be formed from anthranilic acid, L-phenylalanine, and L-methionine by penicillium cyclopium (15). Similarly, antramycin (see Fig. 1), which is a bacterial product of *Streptomyces refuineus,* contains the basic 1,4-benzodiazepine structure (16).

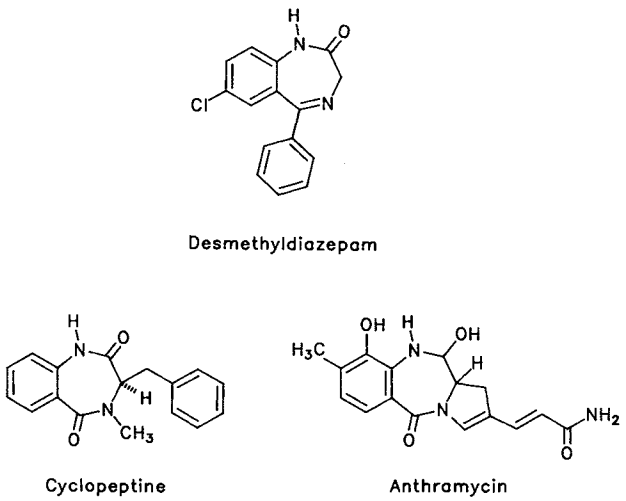

Fig. 1. Structures of desmethyldiazepam (top), cyclopeptine (left), and antramycin (right).

On the basis of our detection of desmethyldiazepam in hen, chicken, and egg white (13), the benzodiazepines appear very early during ontogenesis, and the organism is exposed very early to these drugs during maturation. Dependent on the nature and the amount of food consumption, such indirect administration of benzodiazepines could have some influence on receptor regulation or modification of vigilance and anxiety. However, it must be emphasized that a biological role or the clinical significance of the natural presence of benzodiazepines is still uncertain.

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REFERENCES

1. C. J. Weitz, L. I. Lowney, K. F. Faull, G. Feistner, and A. Goldstein. *Proc. Natl. Acad. Sci. USA* 83:9783–9788 (1986).
2. C. J. Weitz, K. F. Faull, and A. Goldstein. *Nature* 330:674–677 (1987).
3. T. Ubuka, H. Kodama, and S. Mizuhara. *Biochim. Biophys. Acta* 141:266–269 (1967).
4. U. Hannestad and B. Sörbo. *Clin. Chim. Acta* 95:189–200 (1979).
5. H. Frank, D. Thiel, and K. Langer. *J. Chromatogr.* 309:261–267 (1984).

6. L. H. Sternbach. *J. Med. Chem.* 22:1–7 (1979).
7. P. R. Schofield, M. G. Darlison, N. Fujita, D. R. Burt, F. A. Stephenson, H. Rodriguez, L. M. Rhee, J. Ramachandran, V. Reale, T. A. Glencorse, P. H. Seeburg, and E. A. Barnard. *Nature* 328:221–227 (1987).
8. W. Haefely. *Pharmacopsychiatry* 21:43–46 (1988).
9. M. Miyata, I. Mocchetti, C. Ferrarese, A. Guidotti, and E. Costa. *Proc. Natl. Acad. Sci. USA* 84:1444–1448 (1987).
10. L. Sangameswaran and A. L. deBlas. *Proc. Natl. Acad. Sci. USA* 82:5560–5564 (1985).
11. L. Sangameswaran, H. M. Fales, P. Friedrich, and A. L. deBlas. *Proc. Natl. Acad. Sci. USA* 83:9236–9240 (1986).
12. J. Wildmann, H. Möhler, W. Vetter, U. Ranalder, K. Schmidt, and R. Maurer. *J. Neurol. Transm.* 70:383–398 (1987).
13. E. Unseld, D. R. Krishna, C. Fischer, and U. Klotz. Submitted for publication.
14. W. Haefely, E. Kyburz, M. Gerecke, and H. Möhler. *Adv. Drug Res.* 14:165–322 (1985).
15. M. Gerlach, N. Schwelle, W. Lerbs, and M. Luckner. *Phytochemistry* 24:1935–1939 (1985).
16. W. Leimgruber, A. D. Batcho, and F. Schenker. *J. Am. Chem. Soc.* 87:5793–5795 (1965).
17. J. Wildmann, W. Vetter, U. B. Ranalder, K. Schmidt, R. Maurer, and H. Möhler. *Biochem. Pharmacol.* 37:3549–3559 (1988).