INTERACTION BETWEEN DIAZEPAM * AND SEROTONIN **

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The direct chemical interaction between drugs affecting the central nervous system and chemical transmitters is currently studied in our laboratory and has been discussed extensively by one of us [1] for explaining the mode of action of a synaptolytic agent. Such an approach to the study of the psychotropic action of drugs follows the electronic or submolecular concepts recently introduced into biochemistry by Szent-Györgyi [2] and other authors [3].

Serotonin, a central chemical transmitter utilized in the neuron system that is involved in consciousness and affective states, has been recognized as a potent electron donor according to molecular orbital calculations [3]. On the other hand, diazepam (nonproprietary name for 7-Cl-1, 3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) appears to be endowed with the properties of a good π-type electron acceptor [4]. This compound belongs to a class of therapeutic agents which produce tranquilization and exhibits a number of neurologic and autonomic effects [5]. A physiological localization of diazepam effects at the level of the limbic system and its possible interaction with the brain amine metabolism has been put forward, even though no inhibition of the monoaminoxidase system has been observed.

In this communication we wish to report the existence of an interaction between serotonin and diazepam which is demonstrated by means of the differ-

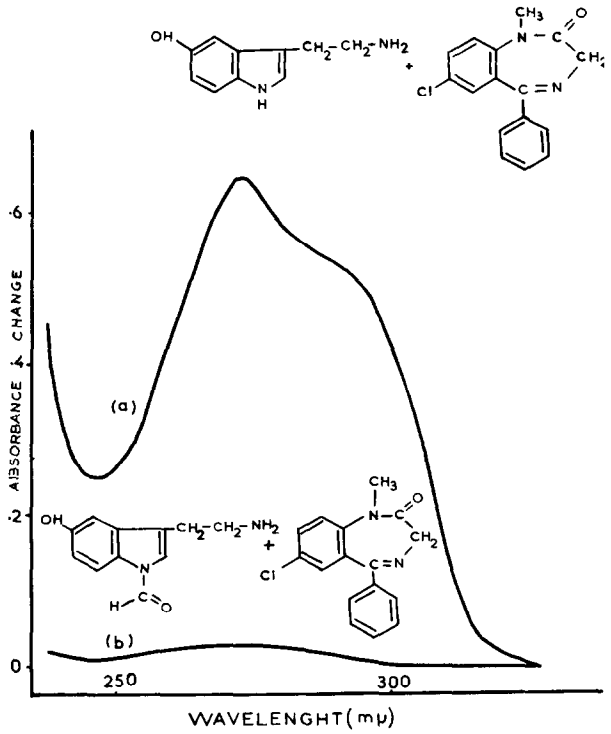

Fig. 1(a). 1 ml of diazepam (Ravizza) 0.1 M in dioxane-water (1:1) is mixed with 1 ml of serotonin oxalate (Calbiochem) 0.1 M in phosphate buffer 0.1 M pH 6.8 and the mixture is diluted 1:10,000 with water before the spectral analysis. The difference spectrum is obtained with a Cary 14 spectrophotometer by placing the serotonin-diazepam mixture in the reference chamber and the two separated solutions of diazepam and serotonin in the sample chamber. In this way a positive peak is obtained as a result of an hypochromic effect. (b) The difference spectrum is obtained following the same procedure described for (a), by using 1-formylserotonin and diazepam.

* The active ingredient of Noan ®, kindly made available by Ravizza, Milano.

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ence spectroscopy technique, and the effect of a chemical modification of the serotonin molecule on its ability to interact with diazepam. Serotonin was subjected to 1-formylation according to the formic acid – hydrochloric acid procedure described by Previero et al. [6] in order to obtain a serotonin derivative in which the donor strength of the π-system is decreased by the introduction of an electron-withdrawing substituent at the indolic =NH group.

The difference spectrum shown in fig. 1a indicates a marked hypochromic effect and a small red shift induced by diazepam on the absorption peak of serotonin. Such an effect certainly is not due to charge-transfer absorption since a new charge-transfer band does not appear whereas a π-π^* type donor-acceptor interaction is very likely. This interaction should give rise to a serotonin-diazepam complex where the resonance contribution to the overall stability is less than that due to other factors such as dispersion forces and electrostatic interactions.

A double reciprocal plot of 1/(diazepam) *versus* 1/difference extinction yields a straight line when the serotonin concentration greatly exceeds the diazepam concentration [7]. Under these conditions an apparent affinity constant of 12×10^3 liters · $Moles^{-1}$ can be calculated, which indicates very strong complexing. The competition with the solvent however was not taken into account and the value can be considered only as a very approximate one.

Our results indicate the formation of a stable complex between serotonin and diazepam. In such a complex serotonin must act as an electron donor and it is very likely that the molecular region with the highest frontier electron density at the No. 2 carbon atom in the indole nucleus [3] is the most important for the formation of a donor-acceptor complex. The formylation reaction represents a good way of deactivating such a region. If one considers the contribution of the electrons of the substituent in modifying the polarizability of the molecule it seems probable that the stabilization of the complex mainly results from electrostatic and Van der Waals forces.

The ability of diazepam in forming molecular complexes with other chemical transmitters such as norepinephrine and acetylcholine was also checked in the present study. A weak interaction was proved by difference spectroscopic analysis because a hypochromic effect and a band broadening were observed in both diazepam-norepinephrine and diazepam-acetylcholine interaction. These effects were observed for the norepinephrine chromophore absorption at 280 mμ and for diazepam chromophore absorption at 310 mμ in diazepam-norepinephrine and diazepam-acetylcholine solutions respectively. These interactions however are much less significant than the serotonin-diazepam interaction, thus allowing one to consider the latter as the one of more probable physiological significance.

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