PROCESS OF INVESTIGATING THE BONDING AND COMPETING PROPERTIES OF BUSPIRONE AND DIAZEPAM IN THE PRESENCE OF RADIOLABELLED LIGAND ([^{3}H]-FLUNITRAZEPAM).

Using radiolabelled Flunitrazepam, the binding properties of Diazepam and Buspirone on the benzodiazepine sites on the $GABA_A$ receptors were explored. Quantitative analysis was accomplished using displacement curves and calculation of IC_{50} values.

Introduction

Radiolabelled ligand binding method has been employed in vivo and in vitro as an indication of power of its measurability. Radiolabelling ligand analysis in terms of binding properties necessitates measurements of free ligand concentration (L_f) and broad-spectrum binding (L_{ns}) to ensure absolute assessment of specific binding. For this laboratory, the potent anxiolytic Flunitrazepam was radiolabelled with Tritium on homogenates of rat cerebral cortex. Therefore, the inhibitory features are related to the techniques of forming bonds with particular benzodiazepine locations rather than incompatible results anticipated by inhibitory binding. The quantity of displacement characteristics was determined. Through this method, one is able to measure the radioactive properties of the assays while exposed to changing Diazepam or Buspirone concentrations. Thus, percentage binding can be computed as quantified radioactivity compared to total specific binding.

N.B. It was determined that the total specific binding by soaking the tissue in Clonazepam can bind to the receptor site with higher affinity. Consequently, a simple subtraction of general from total binding was undertaken.

The anxiolytic and hypnotic drugs debated result in a decline in cerebrocortical norepinephrine discharge. Auto-radiographic diagnostics established that type 2, benzodiazepine kind, receptors in the brains of rats were determined evenly with a slightly greater detection present in the hippocampus. Investigation found that the type 1 receptors are mainly in the cerebellum and substantia nigra and cerebellum, with detection still primarily present in hippocampus. Also, investigation revealed that the type 2 receptors were the cause of hypnotic and anxiolytic symptoms. Type 1 is deemed to have less pharmacological specificity.[1] The $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the

brain of a mammal. The framework of the various isoforms is presented as five matching subunits locked in the form of a pentamer to create a chloride passage. The pore chooses a single chloride ion and is regulated by the existence of the γ-amino carboxylic acid GABA.[1] However, the biological pH in this area implies that the acid form is non-persistent and therefore, the region is dominated by carboxylate species. The tentative antagonistic assays structure indicated the binding specificities of Buspirone and Diazepam on $GABA_A$ receptors in the cerebral cortex of rats and thus, explains the principles of competitive binding. A computer calculated IC_{50} value will be estimated as a constant of dissociation, K_D.

Results

Figure 1: Percentage binding of radiolabeled ligand (2 nM) [^{3}H]-Flunitrazepam when exposed to fluctuating concentrations of Diazepam.

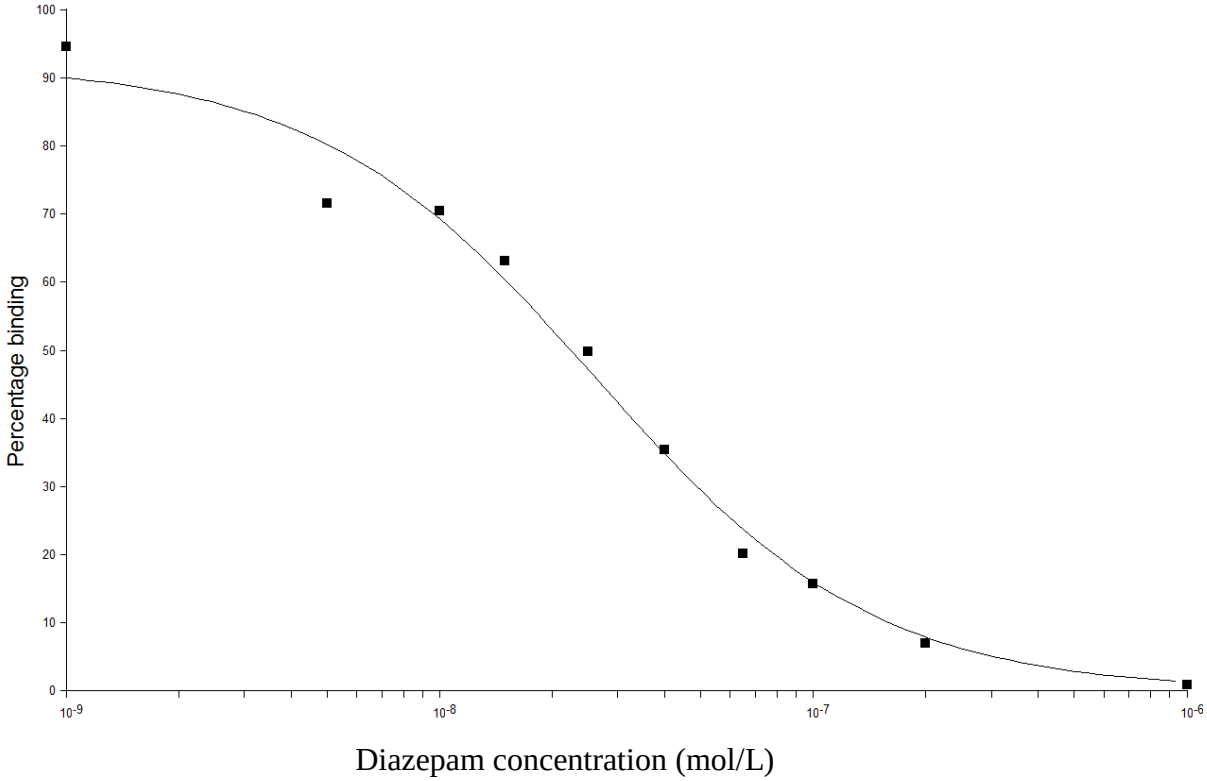

Figure 1 illustrates representative displacement of a ligand over changing concentrations. The initial slow decline in binding is cause by the deliberate kinetics at lesser

concentrations of about < 2nM analogous to the Flunitrazepam molarity. The rest of the curve is a strident reduction in the 10-100 nM scope and propensity to zero.

Figure 2: Percentage binding of radiolabeled ligand (2 nM) [^{3}H]-Flunitrazepam when exposed to varying concentrations of Buspirone.

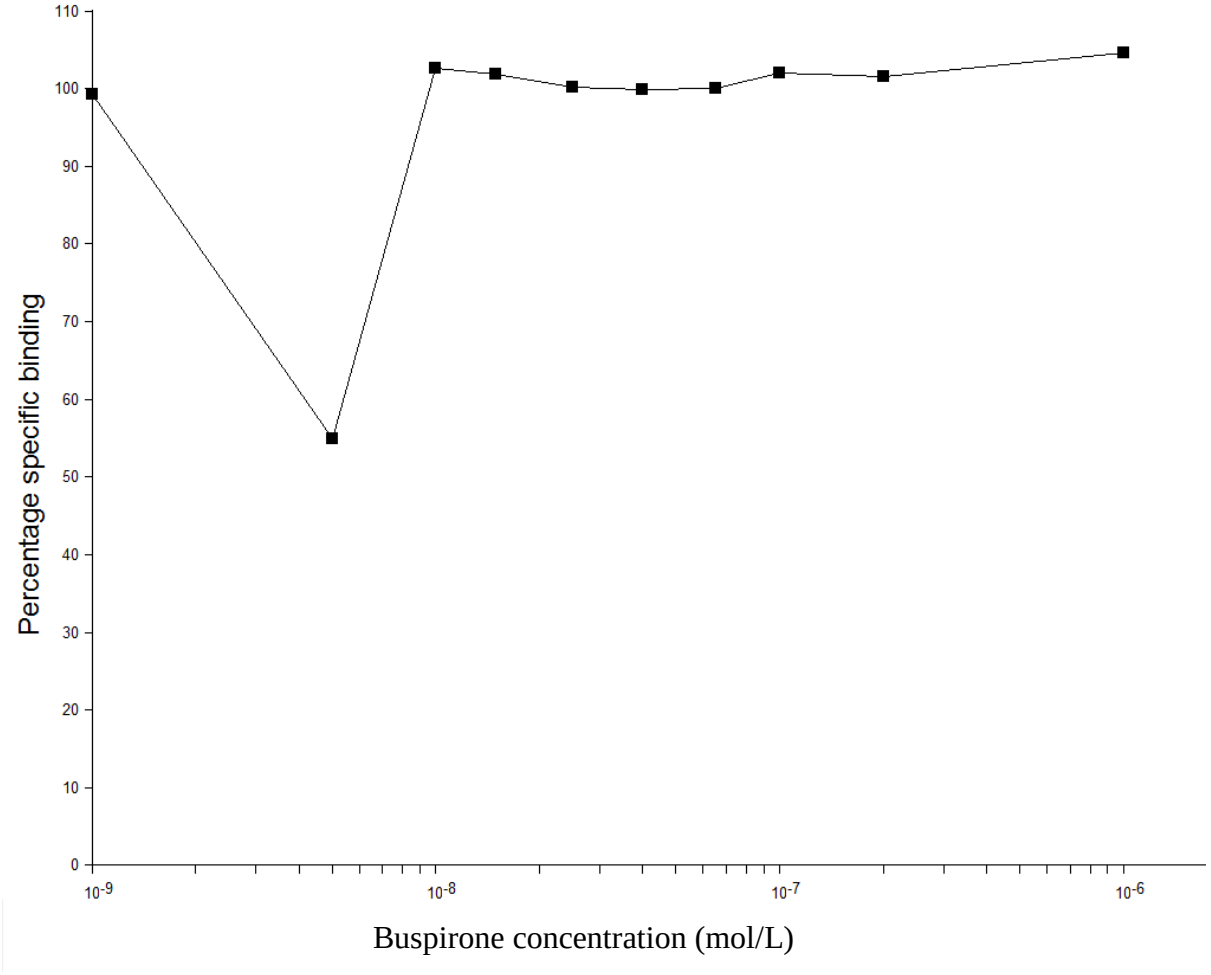

Figure 2 shows that Buspirone is low in competitivity. The graph represents a straight line, which is an indication that a rise in the concentration of radiolabeled ligand has no effect on the percentage binding of the Flunitrazepan. The error point at ~5 nM is, probably, a result of inaccurate distribution of the radio-active drug i.e. < 2 nM.

Figure 3: Saturation assay of a radiolabeled ligand [X] in the homogenate tissue of a rat when exposed to varying concentrations of X to measure ligand binding. The red squares represent ileum tissue, while the black squares represent atria tissues.

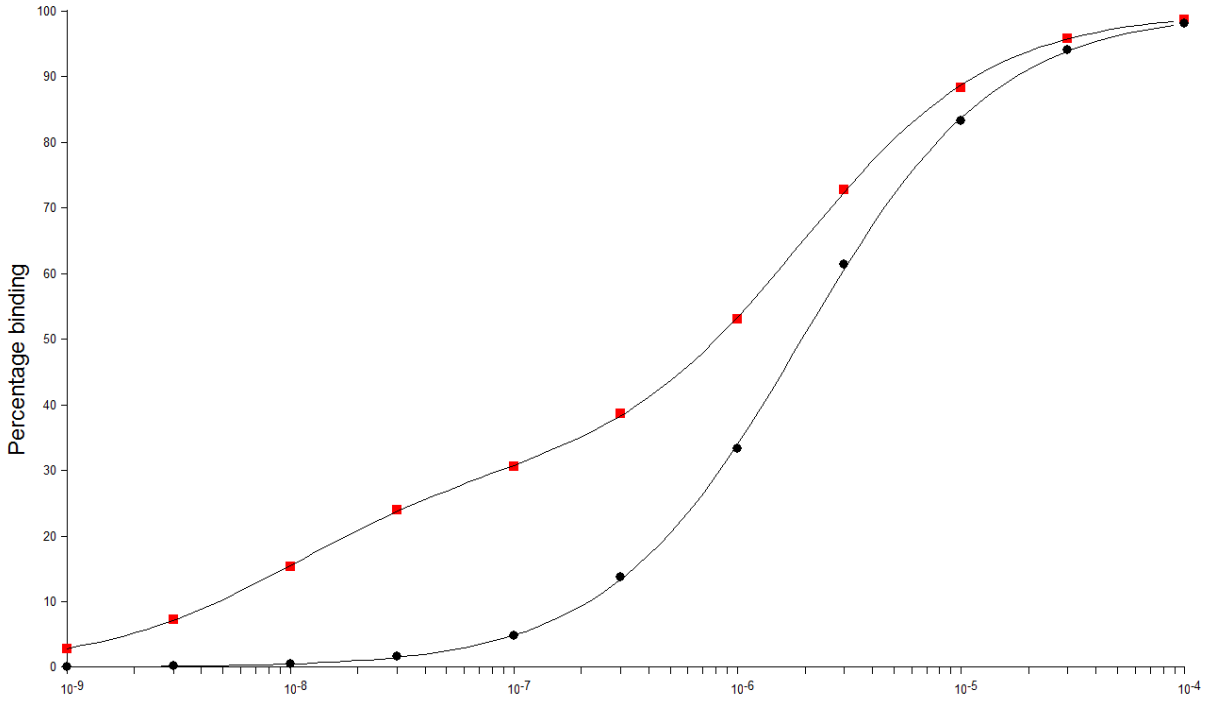

Figure 3 illustrates two diverse types of competition. The values of IC_{50} were calculated and found to be 1.963 µM ± 0.0706 µM (for ileum) and 1.940 µM ± 0.0295 µM (for atria). The atria curve indicates a usual upturn in binding with rising concentrations and saturation quantities within the range of 50 – 100 µM. Comparable features can be seen in the ileum curve, except for a second point of saturation at about 100 nM and approximately twenty-five percent binding indicating binding at two spots binding.

Discussion

We obtained an IC_{50} value of 26.1 nM ± 3.57 Nm from fig 1 quantifying the binding of Diazepam when exposed to Fluritrazepam. Competition with the binding of Fluritrazepam is in the range of 10-100 nM of Diazepam. Thus, the rate of dissociation of Fluritrazepam within this area is higher compared to that of Diazapam. Around the point where the curve starts to decrease rapidly, the concentration of radiolabeled ligand exposed to tissue was 2 nM. It means that the two values of K_D for each of these drugs have to be identical, which is predictable given the similarities in their structure. Fluritrazepam has only a slight divergence compared to the methylated nitrogen present at the amide, which shows a proton. In addition, an atom of Chlorine is interchanged for a nitro group and a Fluorine substituent is added at the 2' point in the benzene ring.[1]

In comparison, Buspirone lacks competitivity, most probably, due to its structure. Buspirone is a longer substrate rich in Nitrogen. Assuming that a specific spot for benzodiazepine type drugs is present, Buspirone may stimulate the $GABA_A$ receptors in a non-specific manner. Also, in terms of structure, the two cyclic areas of this molecule are linked by propyl chain which can add a reasonable suppleness of the substrate that may cause such stimulating effect.

Similarly to Fluritrazepam, Buspirone differs from the normal category of anxiolytic drugs. A variation is present in its biomechanics and clinical countenance. The drug is a 5-HT subtype substrate of serotonin receptors which does not interrelate with the GABA type receptors. Diazepam and other benzodiazepines are good anxiolytics, but they are accompanied by other harmful outcomes such as psychomotor, dependency, and cognitive dysfunction. Buspirone is effective in relieving anxiety without the discussed side effects of common benzodiazepines.[1]

A characteristic saturation curve for a two spot binding is illustrated in the figure 3, and one is able to clearly note a change in the type of receptor in the rat ileum pertaining to atria. Considering a radiolabeled ligand [X], one is also able to note the escalating vulnerability of the ileum. Quicker inclusive mechanics of [X] takes place due to this 'additional site' as a result of its saturation prior to binding on the typical receptor site. There is a similarity in the total saturation, which indicates that the common site is less prevalent in the ileum and is backed by the 'additional site.' In reality, these spots can be pronounced as points of high affinity. Scholars argue that histamine receptors located at the cardiac and bovine vascular tissues can separate various 2-phenylhistamine class competitors. Furthermore, it is specified that 2-phenylhistime equivalents are best positioned to 2-site histamine type receptors.[1] With respect to figure 3, effects of high competitiveness and consequently high specificity of spots in the rat ileum achieve nearly twenty-five percent of total saturation. Taking into account the reduced concentration, reaction occurs due to the greater specificity receptor spot. In terms of atrial saturation, the ileum requirement of [X] would clearly indicate an increased throughput in vivo.

Considering the presence of allosteric variations in the protein content of receptors, they enable the saturation to occur. Hypothesising, once the ~ 25 % saturation of considerably specific binding occurs, conformational alterations could be experienced, permitting less specific binding to occur. Ergo low insistent concentrations of [X] enable proceeding biochemistry to take place at the ileum and to a lesser amount in the atrial areas. It would give room for base levels of [X] to become predominant in flow, hence, ileum function does not stop even with the lack of atria action. Therefore, the changes in structure of Buspirone and Diazepam that permit same anxiolytic side effects indicate that in other circumstances, groups of identical receptors are stimulated by matching substrate.

Differences in structure enable events that depend on concentration to take place independently at ileum and atrial tissue regions.

In recent years, the complex nature of the GABA type receptor, both in terms of subtype heterogeneity and its numerous networking binding spots, has become obvious. Mutagenesis methods as well as biochemical and electrophysiological examination help to have an insight into the structure function associations of this significant neurotransmitter receptor. Using information from research on the GABA group, there have been attempts to create models of receptor-ligand associations and evaluate their results on channel action. Whereas these models offer a convenient context for understanding receptor's structure and function, none can resolve the entire set of experimental data related to gating and binding the GABA. At this instance, a model that explains the intricate allosteric interactions that take place at this receptor is considerably practical.