The Effect of Org. GB 94, Imipramine and Chlorpromazine on the Concentrations of Some Amino Acids in 9 Regions of the Rat Brain

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The concentrations of 11 amino acids were determined in 9 regions of the rat brain following the acute administration of the antidepressant drugs imipramine and Org. GB94, and chlorpromazine. The amino acids were determined as their dansyl derivatives. Unequivocal changes were only found in the concentrations of γ-aminobutyric acid, alanine, asparagine + glutamine, glycine and taurine in some brain regions. Chlorpromazine produced the greatest change in these amino acids. Imipramine and Org. GB94 had qualitatively different effects on the concentrations of the amino acids in the 9 brain regions.

We have previously shown that amino acids, as their dansyl derivatives, can be estimated in small regions of the rat brain by two dimensional chromatography on polyamide plates [1]. Difficulties arise, however, in the quantification of the amino acid concentrations due to the fact that not all the amino acids present in the tissue extract are completely dansylated [2]. This problem has been the subject of detailed comment elsewhere [3]. Although it has been shown that the sensitivity (down to 10^{-14} M) of detection is increased by using [^{14}C]-dansyl chloride we now find that the reproducibility and quantifiability of the method is increased by using unlabelled dansyl chloride; the dansylated products are identified by flluorescent light removed from the polyamide plate by means of micro knives and the products eluted directly in the micro cuvettes by methanol: dilute ammonia solution. The dansylated amino acids are then estimated spectrophotofluorimetrically. We find that this method enables several amino acids to be determined down to a concentration of 10^{-11} M. In the present investigation, the effect of a new anti-depresent drug, Org. GB94 [4] imipramine and chlorpromazine were studied for their effects on the amino acid concentrations in 9 regions of the adult rat brain. Groups of male albino rats (130 – 150 g) treated with physiological saline (control), Org. GB94 (40 mg/kg), imipramine (20 mg/kg) or chlorpromazine (10 mg/kg) were killed by decapitation after 1 hr., the brains rapidly removed, placed on ice and dissected into the neocortex, limbic cortex, olfactory lobes, thalamus, hippocampus, striatum, septum, brain stem and cerebellum. The brain areas were immediately frozen on solid carbon dioxide.

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Small pieces (10 – 30 mg) of the areas were homogenized in 0.05 M sodium bicarbonate solution (pH 10.0), centrifuged (18 000 × *g* for 10 min) and the clear supernatant transferred to small centrifuge tubes. An equal volume of cold acetone was then added to the supernatant and, after mixing, the solution was kept at – 20 °C for 1 hr. After further centrifugation 4 µl aliquots of the supernatant were added to small tubes containing 4 µl of dansyl chloride in acetone (1 mg/ml). After thorough mixing, the tubes were incubated at 37 °C for 30 min, exaporated to dryness *in vacuo* and the dansylated products re-dissolved in 4 µl of acetone: glacial acetic mixture (3:2 v/v). The dansyl amino acids were separated on 3 × 3 cm polyamide sheets using formic acid: water (3:100 v/v) in the first dimension and benzine: acetic acid (9:1 v/v) in the second dimension. After visualization of the products by means of an ultraviolet lamp, 10 dansyl amino acids were reproducibly separated so that the area which the spots occupied could be easily marked and removed from the plate by means of micro knives of the type described elsewhere [7]. At least 30 dansylated products can be separated on micro polyamide plates in this way but it was found that only γ-aminobutyric acid, alanine, glutamine + asparagine, glycine, taurine, proline, methionine, tyrosine, leucine and histidine were separated sufficiently to allow their quantitative removal from the plate and quantitative detection by unlabelled dansyl chloride while ensuring no contamination by adjacent products.

Of the amino acids which were removed in this way, dansyl-taurine was slightly contaminated with dansyl-OH. We have not found it possible to completely separate the amino acid from this contaminant but visual observation of the plates following the 2 dimensional chromatography and a semi quantitative estimation of the amount of dansyl-OH which occurs at the position of dansyl-taurine when a mixture of amino acids, not including taurine, are separated, suggests that this contamination is less than 10% of the total fluorescence for the dansyl-taurine spot.

The area of the polyamide plate containing the dansyl amino acid was added to a micro curvette (volume 0.5 ml) and eluted by a methanol: ammonia: mixture (19:1). It was not necessary to remove the polyamide foil from the micro curvette following the elution of the amino acids as this did

not obstruct the light path during the spectrophotofluorimetric detection of the dansylated product.

To ensure that the 10 amino acids could be quantitatively detected, different concentrations (0.5 – 2.5 μg) of the individual amino acids were added to the supernatant fraction obtained from brain extract and carried through the complete procedure. The recovery (90 – 95%) was determined by addition of commercially dansylated amino acids (Sigma) in the appropriate concentrations. It was found that all 10 amino acids gave a linear increase in fluorescence over the concentration range used; this range approximated to that found in all brain areas studied. In all experiments, the appropriate commercial standards were separated in parallel to the amino acids in the brain extracts and the concentration of the amino acid in the different brain areas calculated in terms of the added dansylated standard.

The results of these experiments are shown in Figs 1 – 4. In addition, the following amino acids were also detected but no significant difference was found between the drug treated and saline treated rats: proline, methionine, tyrosine, leucine and histidine. Of the 5 amino acids determined, glycine, taurine, γ-amino butyric acid and alanine may function as excitatory (alanine) or inhibiting transmitters in different regions of the brain [5, 6].

From these results, it can be seen that the drugs induced changes particularly in the concentration of γ-aminobutyric acid; Org. GB94 caused a rise in the concentration of this inhibitory amino acid in the septum while imipramine caused an increase in the striatum and chlorpromazine raised it in the hippocampus and cerebellum. Chlorpromazine was the only drug which affected the concentration of alanine; its concentration was decreased in the striatum. This drup also increased the glutamine + asparagine in the septum and thalamus whereas imipramine and Org. GB94 were without effect on these amides. Org. GB94 was unique in its action on the inhibitory amino acid glycine; the concentration was decreased in the thalamus. Taurine, which has been implicated as an inhibitory transmit-

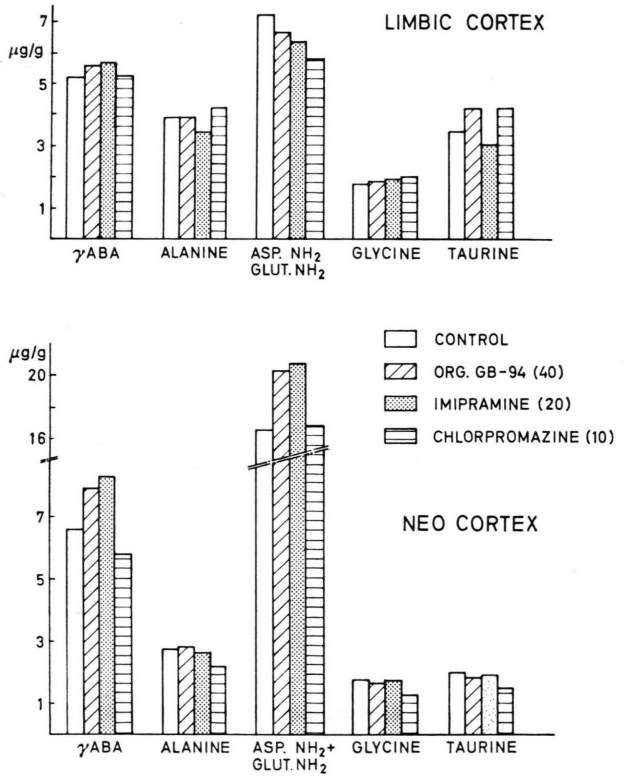

Fig. 1.

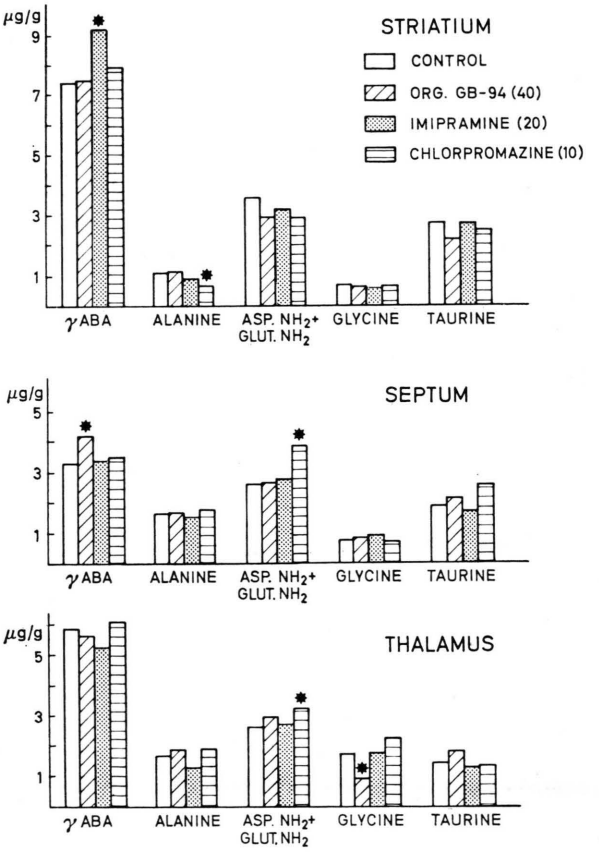

Fig. 2.

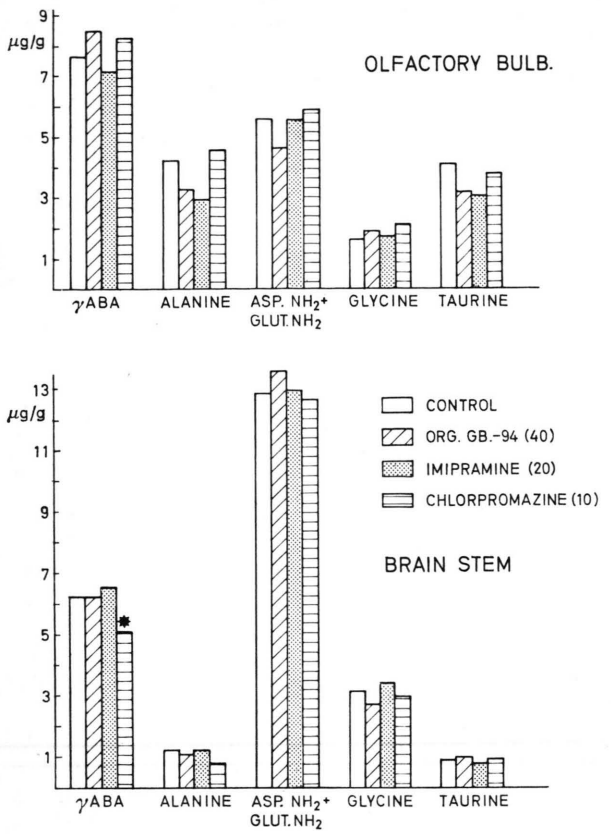

Fig. 3.

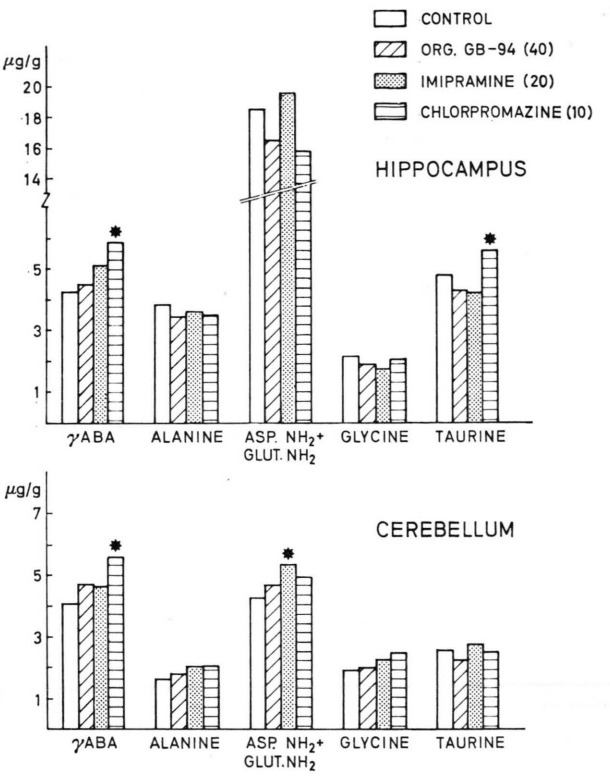

Fig. 4.

Figs 1—4. Concentrations of some free amino acids in 9 regions of the adult rat brain. Each estimation determined in triplicate; minomum of 4 rats used for each treatment group.

* Significance of difference between control and drug treated group $P < 0.05$.

γaba, amino-*n*-butyric acid, $aspNH_2+glutNH_2$, asparagine + glutamine.

ter substance in some regions of the mammalian brain [8], was increased in the hippocampus of rats treated with chlorchromazine. None of the drugs had any apparent effect on the amino acids estimated in the limbic cortex or neocortex.

From this preliminary study it is not possible to draw any conclusions as to the relationship between the therapeutic effects of the 3 neuroleptic drugs and their effects on excitatory and inhibitory amino acids in the 9 regions studies. It is clear, however, that chlorpromazine has a more pronounced effect on these amino acids than the 2 antidepressant drugs. Clearly a more detailed study will be essential before it can be decided whether the pharmacological action of these drugs is partly due to their effects on amino acid transmitter substances in different regions of the brain.

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