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THE INFLUENCE OF HALOPERIDOL AND CHEMICALLY RELATED NEUROLEPTICS ON FREE AMINO ACID CONTENT OF RAT BRAIN

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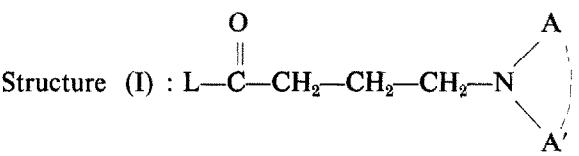
Abstract—The influence of a single subtoxic subcutaneous dose of Haloperidol and of three other neuroleptic drugs of the butyrophenone series on the concentrations of a series of free amino acids occurring in rat brain has been studied. Various significant effects were found. It is doubtful however whether these effects are related to the biochemical mechanism of action of these compounds. A peptide occurring in normal rat brain was found to contain seven different amino acids.

INTRODUCTION

IN RECENT years a large number of basic butyrophenones of general structure (I) were synthesized and investigated pharmacologically in this laboratory.

Structure (I) : 

L = phenyl, substituted phenyl or 2-thienyl.

NAA′ = heterocyclic tertiary amine, such as 4-substituted piperidine or 4-substituted piperazine.

It was found that many of these compounds display strong and typical neuroleptic activity. Some of them are powerful blockers of epinephrine. Their properties are, generally speaking, rather similar to those of chlorpromazine and related phenothiazines.[1–7]

For a number of reasons we became interest in exploring the possible interactions between these butyrophenones and gamma amino butyric acid (GABA):

(1) As shown in Table 1, the structural requirements for GABA-like activity on the one hand and neuroleptic activity on the other hand are strikingly similar.

(2) GABA is known to play an important role as an inhibitor of the central nervous system, particularly of the mid-brain, while neuroleptics must also be regarded as inhibitors of mid-brain function.[18, 19]

The purpose of this paper is to present preliminary results obtained from experiments designed to analyse the influence of various butyrophenones of structure (I) on the concentrations of free amino acids in rat brain.

METHODS

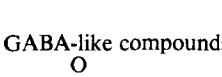
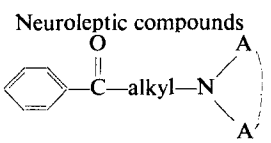
1. *Preparation of the brain extracts*

Untreated male rats of an inbred Wistar strain were used in all the experiments. The pharmacological compounds under investigation were injected subcutaneously

and 4 hr later the rats were killed by decapitation. Control rats, injected with the same amount of solvent were killed at the same time.

The animals weighed around 300 g and were approximately 2½ months of age. The average weight of the brain as determined immediately after removal was 1·6 g. The brains of two rats, injected with the same compound, were pooled and immediately put in 12·8 ml of 75% ethanol per g fresh tissue. Tissue extracts were prepared free of proteins and lipids as described by Roberts[8].

TABLE 1. STRUCTURAL REQUIREMENTS OF GABA-LIKE ACTIVITY AND OF NEUROLEPTIC ACTIVITY

Alkyl	GABA-like compounds 		Neuroleptic compounds 	
	Name	Activity*	Name	Activity†
$-CH_2-$	glycine	+ (1/1300)	α-aminoacetophenones	+ (<1/100)
$-(CH_2)_2-$	β-alanine	+++ (1/20)	β-aminopropiophenones	+++ (1/100)
$-(CH_2)_3-$	GABA	+++++ (1)	γ-aminobutyrophenones	+++++ (1)
$-(CH_2)_4-$	δ-aminovaleric acid	+++++ (1/15)	δ-aminovalerophenones	+++++ (1/10)
$-(CH_2)_5-$	ε-aminocaproic acid	++++ (1/100)	ε-aminocaprophenones	++++ (1/50)
$-CH_2-CH(CH_3)-$	β-aminobutyric acid	++ (1/500)	β-aminobutyrophenones	++ (<1/100)

* Edwards.[17]
† Janssen *et al.*[3–5]

The method of Roberts was slightly modified. The aqueous ethanol extract was evaporated almost to dryness, taken up in 5–10 ml water and centrifuged for 60 min at 6000 rev/min (swing-out heads). The supernatant was again evaporated to ±0·5 ml. By adding water a final volume of 1 ml was obtained.

2. *Chromatography*

Two-dimensional descending chromatography was used. Twenty microlitres of the extract (an aliquot corresponding to 65 mg fresh brain tissue) are placed on Whatman no. 1 paper (35 cm × 35 cm). The first solvent was a mixture of 80 g melted phenol, 20 g water and 10 mg EDTA. After the phenol run, the papers were dried for 1 hr at 65 °C in a "chromatography drying oven" with forced air circulation.

The second solvent was the supernatant of 1 vol. of collidine, 1 vol. of lutidine and 2 vols. of water. The sheets of paper were dried for 30 min at 65 °C. Even better

separation of amino acids was obtained when the papers were first run with phenol and dried at 65 °C before the amino acid mixture was applied.*

3. *Colour development*

The spots were visualized by dipping each sheet of paper in 50 ml of 1% ninhydrin solution in acetone for 10 sec, followed by drying for 30 min at 65 °C.

4. *Elution of the spots*

Quantitative determinations of the coloured spots were performed by cutting out equal strips from the chromatograms and transferring them into test tubes with 1 ml, 2 ml, 5 ml or 10 ml of a 71% methanol solution in water according to the intensity of the spot.[9] Half an hour later the tubes were shaken for 10 sec and the intensity of the colour was measured by differential colorimetry.

5. *Identification of a peptide*

Several spots of a peptide found on different chromatogram were cut out under a u.v. lamp after the papers were heated for 15 min at 90 °C. After eluting the peptides from the pieces of paper by dipping in warm water, the volume of the aqueous extract was reduced to $\pm 0{\cdot}5$ ml by evaporation on a warm water bath. The peptide solution was transferred in a small pressure tube and hydrolysed with the same volume of hydrochloric acid for 24 hr at 110 °C. The tube was opened, HCl evaporated and a two-dimensional chromatogram run with and without addition of known amino acids.

RESULTS

A solution was prepared which contained 10 μmole/ml of aspartic acid, glutamic acid, α-alanine and γ-aminobutyric acid. The amino acids of this mixture were separated by the method as previously outlined.

The absorbances of the eluates from the spots were plotted against the micromoles of amino acid applied to the chromatogram. Fig. 1 shows that Beer's law is followed in the range studied.

Determinations of the concentrations of aspartic acid, glutamic acid, α-alanine and γ-aminobutyric acid per 100 mg fresh brain tissue gave the following results:

Compound	Pairs of rats	Concentration m-mole/100 g fresh brain Average	Range
Aspartic acid	10	0·34	0·30 – 0·38
Glutamic acid	10	1·06	0·92 – 1·12
α-Alanine	10	0·084	0·067– 0·11
GABA	10	0·38	0·36 – 0·44

The following free amino acids in brain tissue could be detected: Intense spots were given by glutamic acid, GABA, aspartic acid, taurine and glutamine; Another intense spot was given by the peptide; Glutathion, glycine, α-alanine, serine, threonine, cystine gave spots of weak colour intensity; Only traces of tyrosine, valine, leucine and β-alanine were found. The peptide disappeared after hydrolysis and was found to contain leucine, valine, tyrosine, α-alanine, glutamic acid, glycine and aspartic acid.

* We wish to thank Dr. Wayne Albers of "The National Institute of Health" (Bethesda) for his advice.

The average R_f-values of the peptide in the two solvents used were 0·24 in phenol and 0·07 in collidine–lutidine.

The influence of relatively large doses of R 1625 (20 mpk), R 2498 (10 mpk), R 2028 (80 mpk) and R 1647 (160 mpk) on the concentration of a series of free amino acids in rat brain was investigated as described in the experimental part. The chemical structures and pharmacological properties of the investigated compounds are given in Table 2.

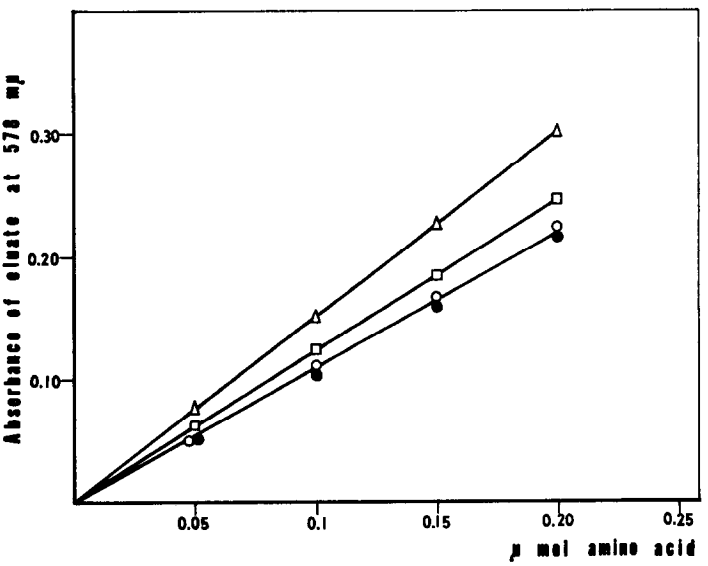
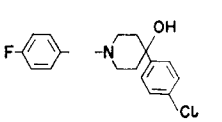
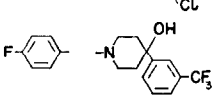
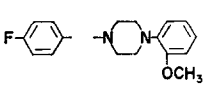
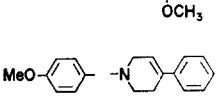


FIG. 1. Absorbance of ninhydrin reaction products as a function of the amount of compound applied to the chromatogram: △ glutamic acid, □ α-alanine, ○ aspartic acid, ● γ-aminobutyric acid. Each mark is the average of five determinations which did not differ more than 10 per cent, respectively.

TABLE 2. CHEMICAL STRUCTURES AND PHARMACOLOGICAL PROPERTIES OF THE INVESTIGATED BASIC BUTYROPHENONES OF STRUCTURE (I)

L- -NAA′	Serial number generic name and references	Neuroleptic activity*	Adrenolytic activity*
	R 1625 Haloperidol[3, 4]	+++	(+)
	R 2498 Triperidol[10]	+++(+)	(+)
	R 2028 Haloanisone[11]	++	+++
	R 1647 Anisoperidone[12]	+	++(+)

* Janssen *et al.*[3–5]

TABLE 3. INFLUENCE OF SUBSTANCES ON CONCENTRATION OF FREE AMINO ACIDS IN THE BRAIN OF RATS

Amino acid	R 1647		R 2028		R 1625		R 2498	
	$f \pm \epsilon$	$P\%$	$f \pm \epsilon$	$P\%$	$f \pm \epsilon$	$P\%$	$f \pm \epsilon$	$P\%$
Glutamic acid	1·03 ± 0·03	50	1·02 ± 0·05	50	1·00 ± 0·03	50	1·03 ± 0·03	50
GABA	1·10 ± 0·03	6·2	1·01 ± 0·02	50	1·00 ± 0·02	50	1·05 ± 0·04	50
Taurine	1·16 ± 0·07	0·8	1·15 ± 0·05	6·2	1·19 ± 0·06	6·2	1·03 ± 0·05	50
Aspartic acid	1·00 ± 0·04	22·7	1·11 ± 0·07	34·4	1·06 ± 0·06	18·8	1·09 ± 0·07	18·8
Glutamine	1·08 ± 0·08	50	1·23 ± 0·04	0·8	1·14 ± 0·03	0·8	1·11 ± 0·08	50
Glycine	1·20 ± 0·05	0·8	1·11 ± 0·08	22·7	1·18 ± 0·06	6·2	0·94 ± 0·04	18·8
Alanine	1·04 ± 0·05	50	1·05 ± 0·03	18·8	1·00 ± 0·06	50	1·09 ± 0·04	18·8
Threonine	1·04 ± 0·06	50	1·21 ± 0·13	22·7	1·00 ± 0·12	50	1·11 ± 0·10	18·8
Serine	1·22 ± 0·09	10·9	1·06 ± 0·05	50	1·15 ± 0·08	22·7	1·55 ± 0·12	3·1
Valine	1·21 ± 0·06	6·2	1·27 ± 0·06	6·2	1·01 ± 0·08	22·7	1·31 ± 0·07	3·1
Leucine	1·35 ± 0·23	22·7	1·11 ± 0·03	1·6	0·82 ± 0·06	1·6	1·13 ± 0·05	3·1
β-Alanine	0·98 + 0·10	50	0·85 + 0·04	6·2	0·93 ± 0·08	10·9	0·91 ± 0·07	50
Glutathione	1·08 ± 0·15	50	1·39 ± 0·08	0·31	1·07 ± 0·18	50	0·94 ± 0·06	50
Peptide	0·94 ± 0·06	50	1·14 ± 0·05	0·31	1·06 ± 0·04	50	1·26 ± 0·08	3·1

The brain extract of both the drug-injected and of the control groups were treated in an identical fashion, 20 μl of each being put on the chromatogram after reduction of the total volume to 1 ml. A total of seven experiments were conducted for each compound. The results are summarized in Table 3, in which the following symbols are used:

(1) $f = \epsilon(x/y)/7$, in which x and y are the extinction values for the treated and the untreated animals, respectively.

(2) ϵ = standard error ($n = 7$) of f.

(3) $P\%$ = the binomial probability of the H_0-hypothesis (one-tailed).

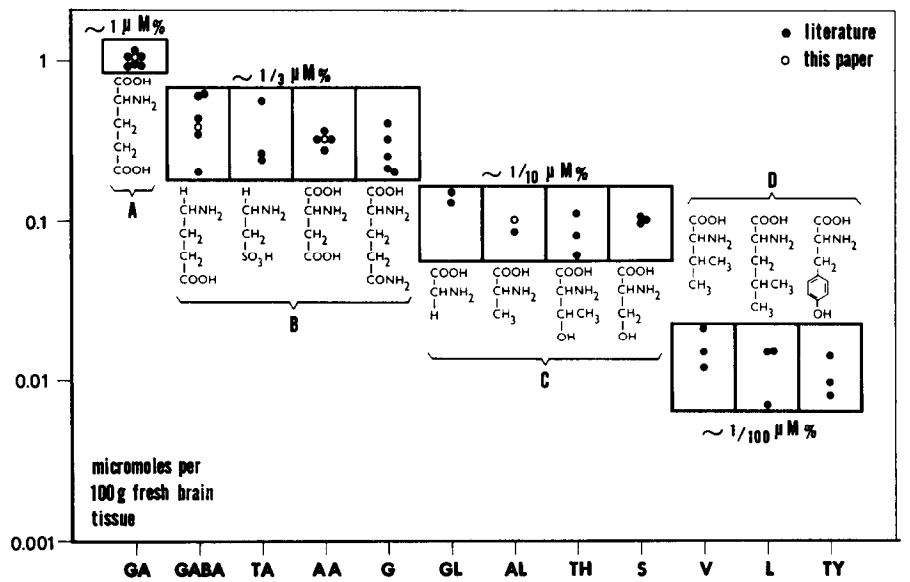


FIG. 2. The table of Porcelatti[13] has been further completed by data from Waelsch[14], Tsukada[15] and Häkkinen[16].

DISCUSSION

As shown in Fig. 2, our findings concerning the free amino acid content (glutamic acid, GABA, aspartic acid and alanine) in the brain of normal laboratory rats, are in excellent agreement with corresponding data from the literature.[13] At least twelve free amino acids occur in rather constant concentration in rat brain. They may be subidivided in four groups, A to D, as shown in Fig. 2. Glutamic acid, the only member of group A, occurs in the amazingly constant concentration of 1·0 $\pm$ 0·1 μmole per 100 g fresh tissue. The concentration of the four members of group B are roughly the same and about 0·4 $\pm$ 0·2 μmole %. The four amino acids of group C occur at a concentration of about 0·10 $\pm$ 0·05 μmole %, and the three acids of group D of about 0·013 $\pm$ 0·007 μmole %. It is interesting to speculate on the biochemical mechanisms responsible for this classification. The peptide which is present in normal brain extracts of rats, was found to contain seven amino acids: glutamic acid (group A), aspartic acid (group B), glycine, α-alanine (group C), valine, leucine and tyrosine (group D). The exact structure, the biochemical and neurophysiological significance of this peptide are to be studied in further detail.

Before trying to interpret the effects of the four neuroleptics studied on the free amino acid concentration of rat brain it should be pointed out that the results of Table 3 were obtained after treatment (4 hr) with a single and very high subtoxic subcutaneous dose of each compound.

In contrast with expectations, GABA was not affected at all after treatment with R 2028, R 1625 or R 2498 and only slightly increased after R 1647.

Glutamic acid, the precursor of GABA, was equally unaffected by all four drugs. Glutamine concentrations, however, were significantly increased after R 2028 and R 1625, but less so after the other two drugs. The other members of group B, i.e. taurine and aspartic acid, also showed a slight tendency to increase, which was significant in some instances. The amino acids of group C were either not influenced or slightly increased in a few cases, i.e. glycine with R 1647 and with R 1625 and serine with R 2498 and possibly with R 1647. A significant increase in valine-concentrations was observed after R 2498, R 1647 and R 2028, whereas leucine was increased after R 2028, R 2498, decreased after R 1625 and not influenced by R 1647. Glutathione, β-alanine and the peptide were also influenced in some instances, particularly by R 2028.

It may be concluded therefore that high doses of all four drugs do have various significant effects on the amino acids in the brain of rats. The whole picture, however, is rather confusing in that the effects vary considerably from compound to compound. This is particularly striking for R 1625 and R 2498. These drugs are very similar pharmacologically and clinically and approximately equi-active. Their effects in the experiments described in this paper, however, are rather different. No useful clue as to a possible relationship between the mechanism of action of these drugs and amino acid metabolism and function can be derived from our experiments. It should be pointed out, however, that our data do not constitute adequate proof of the contrary.

The influence of smaller repeated doses of neuroleptic agents on amino acid metabolism in isolated areas of the brain, e.g. the area postrema, merits further study.

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