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The Effects of Some Derivatives of Gamma-Aminobutyric Acid on the Slowly Adapting Stretch Receptor of *Astacus Fluviatilis*

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Introduction

Recent interest in gamma-aminobutyric acid (GABA) is concerned with its possible role as an inhibitory transmitter substance or regulator of neuronal activity in the central nervous system.[1,2]

Since GABA has been shown to cause slowing of the frequency or abolition of the impulses set up in response to constant stretching of the slowly adapting crayfish stretch receptor [3–5] we considered it of interest to examine the effect of *N*-substituted derivatives of the acid, its esters and amide on this preparation.

The resemblance between crustacean stretch receptor organs and vertebrate muscle spindles has been noted by Kuffler.[6] Both have sensory and motor nerves and respond to stretch in a similar way, but whereas mammalian muscle spindles are modified muscle-cells lying within bundles of ordinary muscle fibres, the muscles of crustacean stretch receptor organs are individual muscle units free from the rest of the musculature. Thus the action of drugs can be studied on the crustacean stretch receptor organs either *in situ* or when removed from the animal.

Methods

Chemical

Compounds hitherto unreported or reported only in low yield were prepared as follows:

Methyl γ-piperidinobutyrate hydrochloride (*DF 494*). γ-Piperidinobutyronitrile[7] (22·7 g) was dissolved in methanol (20 ml) and methanolic HCl (48 ml) was added slowly with cooling, the reaction

mixture being thereafter left at room temperature for $1\frac{1}{4}$ h and refluxed for a further $\frac{3}{4}$ h. On cooling, the solids were dissolved in the minimum of water, and the reaction mixture was basified with 0·880 ammonia and extracted with ether (2 × 50 ml) and chloroform (2 × 40 ml). After removing the dried solvents, the residue was distilled *in vacuo* to give a colourless oil (21·5 g, 78 per cent) b.p. 82–84°/1–2 mm.

The *hydrochloride* was prepared by taking up the ester in isopropyl ether and neutralizing with a solution of hydrogen chloride in isopropyl ether. The solid was thoroughly washed with ether and after crystallization from ethanol–ether had m.p. 146–148°.

Anal. Calcd. for $C_{10}H_{20}ClNO_2$: Cl, 16·02; N, 6·32. Found: Cl, 15·80; N, 6·33.

γ-Piperidinobutyramide hydrochloride (*DF 480*). Methyl γ-piperidinobutyrate (10 g) and 0·880 ammonia (50 ml) were warmed on a steam bath for 8 h, the mixture was evaporated to dryness *in vacuo* and the residue triturated with acetone to give the crude amide (6·5 g, 76·5 per cent), which crystallized from dry acetone as fine needles, m.p. 70–72°. The *hydrochloride* was prepared in dry acetone as above and after crystallization from ethanol had a m.p. 190–192°.

Anal. Calcd. for $C_9H_{19}ClN_2O$: Cl, 17·19. Found: Cl, 16·90.

γ-Aminobutyramide hydrochloride (*DF 510*). Ethyl γ-aminobutyrate HCl[18] (6·1 g, m.p. 72–75°) and 0·880 ammonia (25 ml) were shaken at room temperature in a closed vessel for 3 h, the reaction mixture being thereafter evaporated to dryness *in vacuo* and the residue taken up in 2-propanol. The haze was filtered through kieselguhr and a slight excess of dry HCl gas in 2-propanol was added to the filtrate to give a hygroscopic amorphous solid (1·9 g) which, after three crystallizations from 2-propanol–ether, had a m.p. 126–129° with softening at 115°. The final product (0·68 g, 14 per cent) was also hygroscopic.

Anal. Calcd. for $C_4H_{11}ClN_2O$: Cl, 25·63. Found: Cl, 25·83.

γ-Morpholinobutyric acid hydrochloride (*DF 533*). (*a*) Methyl γ-morpholinobutyrate was prepared in 72 per cent yield from the corresponding nitrile[7] by the method used for the γ-piperidino analogue above. The ester distilled as a colourless liquid b.p. 101–102°/3 mm; n_D^{21} 1·4600. It gave a *hydrochloride*, m.p. 131–133°, after crystallization from amyl acetate.

Anal. Calcd. for $C_9H_{18}ClNO_3$: Cl, 15·88. Found: 15·76.

(*b*) The ester from (*a*) was hydrolysed by refluxing with HCl (18 per cent w/v) for 3 h. The reaction mixture was evaporated *in vacuo* and the residue was crystallized from ethanol to give white rosettes, m.p. 181–183°.

Anal. Calcd. for $C_8H_{16}ClNO_3$: Cl, 16·94. Found: Cl, 16·86.

γ-Butyrobetäine hydrochloride (*DF 574*). Methyl γ-chlorobutyrate[8] (8·2 g) was dissolved in dry acetone (20 ml) and an equimolecular amount of sodium iodide in acetone (10 g in 50 ml) was added at room temperature with stirring. The mixture was refluxed for 1½ h before filtering the NaCl formed and removing the solvent *in vacuo*. The residue was taken up in methanol (20 ml), a 33·33 per cent (w/v) solution of methylamine in methanol (120 ml, 120 per cent excess) was added and the mixture was refluxed for 24 h using a Dry-Ice trap. The quaternary iodide was precipitated quantitatively (10 g, m.p. 135–137°) by addition of excess of dry ether. An aqueous solution of this iodide was then stirred at room temperature for 4 h with a slight excess of silver oxide, filtered, and evaporated to dryness *in vacuo*. The crude betaine (6·2 g) was dissolved in methanol (10 ml), acidified with HCl in isopropyl ether and a small amount of dry ether added to complete precipitation of the hydrochloride. The solid was washed thoroughly with dry ether on the filter and crystallized twice from absolute alcohol to give a white solid (2·2 g, 20 per cent), m.p. 212–214°. (Willstätter describes a method for the preparation of the betaine only.)

Anal. Calcd. for $C_7H_{16}ClNO_2$: Cl, 19·56; N, 7·71. Found: Cl, 19·40; N, 7·50.

γ-Piperidinobutyroylpiperidine hydrochloride (*DF 531*). γ-Chlorobutyric acid[9] was dissolved in benzene (30 ml) and thionyl chloride (30 ml, 100 per cent excess) was added dropwise with stirring over a period of 30 min. The reaction mixture was stirred for a further 2 h and left overnight at room temperature. The excess of thionyl chloride was removed by azeotroping with benzene and the residue was distilled *in vacuo* to give a colourless mobile liquid (14·6 g, 57 per cent) b.p. 40–42°/1·5 mm, n_D^{22} 1·4631. The latter acid chloride (22 g) was dissolved in dry acetone (100 ml) and a solution of piperidine (26 g, 120 per cent excess) in acetone (100 ml) was added dropwise with stirring and

ice-cooling. Piperidine hydrochloride in theoretical quantity was filtered off and the intermediate, γ-chlorobutyroylpiperidine, reacted immediately with sodium iodide (27 g). After standing at room temperature for 2 h, the NaCl was filtered off, piperidine (13·0 g, 120 per cent excess) added and the mixture refluxed for 11 h, cooled and ether added in slight excess to precipitate the piperidine hydriodide. (The amount of ether appeared to be critical; too much gave an oil instead of a crystalline solid.) The hydriodide was filtered off, the acetone removed and the residue fractionated *in vacuo* to give as main product a yellow viscous oil (9·8 g, 30 per cent), b.p. 163°/2 mm, which on redistillation had a b.p. 162°/1·5 mm, n_D^{21} 1·4999. The latter gave a *hydrochloride* which was crystallized twice from amyl acetate to give a white product m.p. 177–178°.

Anal. Calcd. for $C_{14}H_{27}ClN_2O$: Cl, 12·90; N, 10·2. Found: Cl, 12·90; N, 9·74.

Ethyl γ-dimethylaminobutyrate methiodide (*DF 569*). The ethyl ester was prepared from γ-dimethylaminobutyronitrile[7] and absolute alcohol saturated with hydrogen chloride gas using conditions similar to those described for methyl γ-piperidinobutyrate above. The ester was a colourless oil, b.p. 70°/7 mm, and was converted to the crude methiodide (8·6 g) by dissolving in dry ether and allowing the solution to stand at room temperature for 3 h with excess of methyl iodide. After two crystallizations from ethanol, the product (4·5 g, 40 per cent) had m.p. 151–152°.

Anal. Calcd. for $C_9H_{20}INO_2$: I, 42·17. Found: I, 42·01.

The following compounds were prepared by previously described methods:

γ-Aminobutyric acid (DF 468), m.p. 200–202°, from aqueous alcohol (Tafel and Stern[18] gave a m.p. 202°).

Anal. Calcd. for $C_4H_9NO_2$: N, 13·40. Found: N, 13·59.

γ-Acetamidobutyric acid (DF 469), m.p. 126–128° from amyl acetate (Reppe *et al.*[14] give m.p. 129°).

Anal. Calcd. for $C_6H_{11}NO_3$: mol. wt., 145. Found: mol. wt. (by titration), 143·3.

γ-Benzamidobutyric acid (DF 470), m.p. 80–82° from benzene (Todd and Teich[15] gave m.p. 79–80°).

Anal. Calcd. for $C_{11}H_{13}NO_3$: mol. wt., 207. Found: mol. wt. (by titration), 206.

γ-Dimethylaminobutyric acid hydrochloride (DF 471), m.p. 147–149°, from ethanol (Keil[12] gave m.p. 142°).

Anal. Calcd. for $C_6H_{14}ClNO_2$: mol. wt., 167·5. Found: mol. wt. (by titration), 167·0.

γ-Diethylaminobutyric acid hydrochloride (DF 472), m.p. 162–164°, from ethanol–ether (Todd and Teich[15] gave m.p. 166°).

Anal. Calcd. for $C_8H_{18}ClNO_2$: mol. wt., 207. Found: mol. wt. (by titration), 206.

γ-Piperidinobutyric acid hydrochloride (DF 473), m.p. 193–194°, from ethanol (Thomas and McElvain,[16] gave m.p. 189–190°).

Anal. Calcd. for $C_9H_{18}ClNO_2$: Cl, 17·11. Found: 17·10.

Ethyl γ-diethylaminobutyrate (DF 495), b.p. 70–72°/8 mm (Wohlgemuth[13] gave b.p. 98°/13 mm).

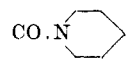
Anal. Calcd. for $C_{10}H_{21}NO_2$: mol. wt., 187. Found: mol. wt. (by titration), 186·7.

Pharmacological

Using the ventral approach, stretch receptors from the second or third abdominal segments of the fresh-water crayfish *Astacus fluviatilis* were carefully dissected out under van Harreveld's crayfish solution[10] by the method described in detail by Wiersma, Furshpan and Florey,[11] and Kuffler.[6] The receptor units consist of a pair of fine muscle bundles in each half of the abdominal and thoracic segments of the crayfish with one muscle finer and shorter than the other. Both muscle bundles are supplied by a common nerve trunk, containing efferent motor and inhibitory nerves, which fans out into separate fibres as it approaches the muscles. Each muscle has its own sensory neurone with its cell body lying close to the muscle and the axon joining the common nerve trunk.

The slowly adapting receptor unit, consisting of the finer of the two muscle bundles together with small pieces of skin and shell to which its attachments were fixed at each end, and its nerve trunk were suspended in a bath of crayfish solution by means of two small clamps which held the shell at each end of the muscle. The nerve trunk was laid over a silver wire electrode and drawn up into a layer of liquid paraffin. Tension was applied to the preparation by moving the clamps apart and the potentials were amplified and

Table I. Chemical and pharmacological properties of some derivatives of gamma-aminobutyric acid

DF no.	X	— $(CH_2)_3$ —	Y	Salt	Concentration, μg/ml	Crayfish stretch receptor: No. of prep. on which tested	Crayfish stretch receptor: Effect on impulse-frequency/sec
471	$(CH_3)_2N$—		COOH	HCl	25–75	2	Increase of up to 4 times initial rate
472	$(C_2H_5)_2N$—		COOH	HCl	100–135	2	Increase of up to 2 times initial rate
469	$CH_3CO.NH$—		COOH	—	150	1	Increase of up to 3 times initial rate
470	$C_6H_5.CO.NH$—		COOH	—	50–80	3	Increase of up to >2 times initial rate followed by sudden abolition of impulses
533			COOH	HCl	30	1	Increase of up to 2 times initial rate
473			COOH	HCl	105	1	Increase of up to 2 times initial rate
494			$COOCH_3$	HCl	85	1	Increase of up to >3 times initial rate followed by sudden abolition
574	$(CH_3)_3N^+$—		COO^-	HCl	10–25	1	Increase of up to 3 times initial rate followed by sudden abolition
468	NH_2		COOH	—	2·5–50	10	Decrease after sometimes slight increase. Often complete abolition
480			$CONH_2$	HCl	12·5–125	2	Decrease after sometimes initial slight increase. Complete abolition in 1 preparation
510	NH_2		$CONH_2$	HCl	50–125	2	Decrease after sometimes initial slight increase. Complete abolition in 1 preparation
531			CO. 	HCl	300	1	No effect
495	$(C_2H_5)_2N$—		$COOC_2H_5$	—	100	1	No effect
569	$(CH_3)_2N$—		$COOC_2H_5$	CH_3I	65	1	No effect

recorded with the aid of an Ediswan pen recorder oscillograph. The usual frequency of the impulses set up after stretching the preparation was from 4–10/sec.

Drugs were added by means of a syringe to the salt solution bathing the preparation.

Results

Detailed results are given in Table I.

Stretching the slowly adapting receptor resulted in a train of impulses which varied in frequency with the amount of tension applied. With a constant tension the frequency often remained unaltered for several hours.

The inhibitory effect of γ-aminobutyric acid on these stretch potentials was confirmed. Concentrations of 2·5 to 50 μg/ml, depending on the sensitivity of the preparation, reduced the frequency or abolished the impulses completely although sometimes a slight initial increase in frequency was observed. With the lower concentrations, spontaneous recovery was obtained if the preparation was left undisturbed (Fig. 1a), but with less sensitive preparations or higher concentrations of GABA recovery could only be obtained after washing the preparation (Fig. 1b).

Substitution at the amino group of GABA by acyl, dialkyl, piperidino and morpholino groups gave compounds which accelerated the frequency of the impulses up to four times the initial rate, as did γ-butyrobetäine. This rapid discharge of the impulses was sometimes followed by sudden abolition, which could only be reversed by washing the preparation (Fig. 2).

The amides of GABA and γ-piperidinobutyric acid decreased frequency or abolished the impulses completely.

Simultaneous substitution of the terminal groups of GABA by two piperidino rings or by diethylamino and ethyl groups had no noticeable effects at the concentrations used, nor had ethyl γ-dimethylaminobutyrate methiodide, the only quaternary salt tested.

No attempt was made to determine the relative potencies of these compounds as the preparations on which they were tested varied considerably in sensitivity.

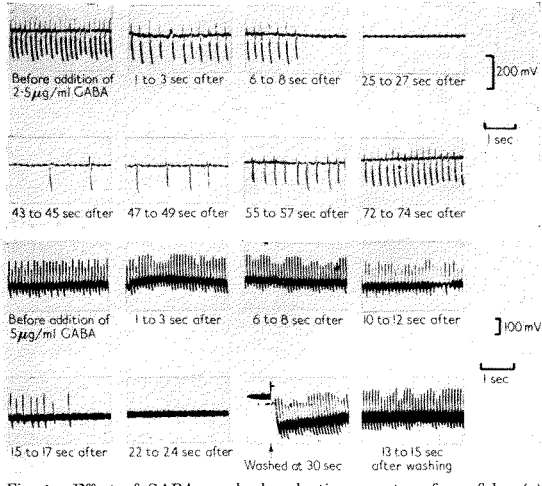


Fig. 1. Effect of GABA on slowly adapting receptor of crayfish. (a) Spontaneous recovery after addition of 2·5 μg/ml. (b) Recovery on washing after addition of 5·0 μg/ml.

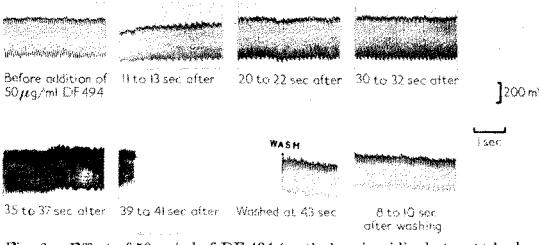


Fig. 2. Effect of 50 μg/ml of DF 494 (methyl γ-piperidinobutyrate hydrochloride) on slowly adapting receptor of crayfish.

Discussion

A possible role for GABA in central inhibitory mechanisms has been postulated,[1,2] but administration of the compound to animals has failed to produce any marked effect except by the intracerebral route.[19] It seems possible that this lack of activity might be due to the failure of GABA to penetrate the blood–brain barrier,[20] or to its rapid destruction, though this appears unlikely as *in vitro* experiments have shown that brain suspensions do not inactivate GABA at an appreciable rate.[21]

The present work was part of a screening programme to determine the possibility of producing compounds with an activity greater than that of GABA on the crayfish stretch receptor preparation, in the hope that such compounds might be effective when administered to animals by routes other than the intracerebral one. Although the findings of earlier workers,[3–5] that GABA caused slowing and abolition of the impulses in response to constant stretch of the slowly adapting stretch receptor, were confirmed, the structural modifications employed did not lead to compounds with greater blocking activity than GABA itself.

Most of the compounds we tested had the effect of accelerating the frequency of the stretch impulse. Edwards and Kuffler[22] noted that several acids of varied structure, such as succinic, α-ketoglutaric and γ-amino-β-hydroxybutyric acid trimethyl betaine, also showed this property. As in their experiments, no attempt was made to determine whether the inhibitory and excitatory substances were acting directly on the nerve or indirectly on the muscle itself.

Summary. The effects of GABA and some *N*-substituted amide and ester derivatives of the acid have been studied on the potentials arising in response to constant stretch of the slowly adapting stretch receptor of *Astacus fluviatilis*. Results indicated that gamma-substitution in the acid increased the frequency of the impulse, in contrast to GABA itself which decreased the frequency, as did the two butyramides tested. Simultaneous substitution at both terminal groups of GABA had no noticeable effect on the impulse frequency.

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