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## The phase reversal phenomenon of the GABA-action on the hind gut of the crayfish caused by the change in the ionic composition of the external medium

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THE ISOLATED crayfish hind gut preparation was first described by Florey[1] and it was recently reported that this new method is simpler and more sensitive[2] than that using crayfish stretch receptors Using the hind gut of Astacus astacus L., which is a different species from that used by Florey, Jones recently found that L-glutamic acid caused contractions.[3]

We confirmed Florey's data using the American crayfish. Actylcholine (ACh) at a concentration of $10^{-7}$~$^{-5}$ g/ml caused contraction, and $\gamma$-aminobutyric acid (GABA) at aconcentration of $10^{-4}$~$^{-5}$ g/ml caused relaxation of the preparation. The ACh-induced contraction of the gut was blocked by pretreatment of the preparation with GABA. We studied the relationship between the action of GABA and the ionic composition of the external medium. The results obtained, are shown in the figure.

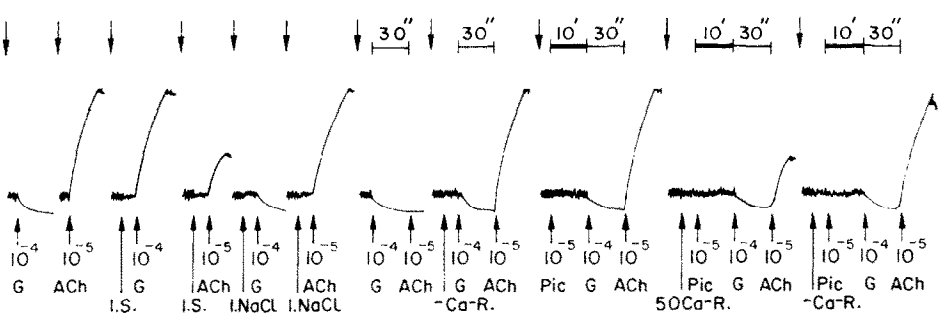


FIG. 1. The GABA and ACh-action on the hind gut of the crayfish caused by change in the ionic composition of the external medium.

The arrows at the top indicate the change of Florey's Ringer solution, and the row of numbers refers to time. The numbers $10^{-4}$ or $10^{-5}$ refer to the dilution. The bottom row refer to the dilution of the external medium.

The abbreviations used are as follows. G: $\gamma$-aminobutyric acid, ACh: acetylcholine, Pic: picrotoxin, I.S.: isotonic sucrose, I.NaCl: isotonic 1·2% NaCl solution, -Ca-R: $Ca^{2+}$ free Ringer solution, 50 Ca-R.: 50% Ca-Ringer solution.

First, the effect of replacing Florey's crustacean Ringer solution by isotonic sucrose or glucose solution as the external medium, was studied. This tended to slow the contractions.

When GABA was added fifteen minutes after replacing the external medium by sucrose, the preparation which had been in a relaxed state in Ringer medium contracted after a short latent period. Because of the short latent period and because this phenomenon occurred in isotonic sucrose medium, this reversal phenomenon is probably not related to metabolism.

These reversals were also observed with many other amino acids with several carbon atoms between the cationic and anionic sites in their molecule, such as, in decreasing order of potency; L-glutamic acid, β-hydroxy-γ-guanidinobutyric acid, L-aspartic acid, β-alanine, threo- and erythro-DL-β-hydroxyglutamic acid, γ-guanidinobutyric acid and β-hydroxy-γ-aminobutyric acid.

The structure-activity relationship of compounds related to GABA tested on the intensity of the reversal phenomenon, was similar to the results obtained in Ringer solution.

To study the reason for this reversal further, crustacean Ringer solution was progressively modified by changing the composition of the various ions, and sucrose was added to maintain osmotic pressure at a constant level.

With these solutions it was found that this reversal appeared when the concentration of sodium was reduced to 60% of that in normal crustacean Ringer solution.

In contrast, reversal appeared in simple NaCl-medium when the concentration of NaCl was lowered to 20% of the initial sodium concentration in the medium.

Cations other than the ammonium ion, such as choline ion or potassium ion blocked the excitability of the preparations. Only the ammonium ion behaved as the sodium ion, and other anions similar to $Cl,^-$ i.e. $Br,^-$ $I,^-$ $NO_3^{2-}$, $SO_4^{2-}$, $SCN^-$, $H_2PO_4^-$ behaved as $Cl^-$ at isotonic concentrations. The effect of hyper- and hypotonic solutions was tested. From the above results, it seems that only $Na^+$ is essential for the GABA-activity of relaxation of the hind gut of the crayfish. Another interesting ion beside $Na^+$ was $Ca^{2+}$. The blocking activity of GABA on ACh-induced contractions of the crayfish intestine decreased with decrease in $Ca^{2+}$ concentration and the blocking activity was almost completely lost in $Ca^{2+}$-free solution.

These results on $Ca^{2+}$-free solutions are closely similar to Florey's data on preparations pretreated with picrotoxin. However, in the preparation pretreated with picrotoxin, the blocking activity of GABA on ACh-induced contraction, increased as the $Ca^{2+}$ concentration was lowered to 50% of the normal concentration, but with further decrease in $Ca^{2+}$ content the blocking activity of GABA on ACh-induced contraction was again lost.

These results suggest that the picrotoxin effect on this preparation is related to depletion of the membrane of $Ca^{2+}$.

There is evidence that $Na^+$ may be closely related to the action of GABA.

We found that the convulsive activity caused by intraventricular injection of GABA, was high when the injection fluid contained 0·45–0·9% NaCl.

Further Sano and Roberts found that only the $Na^+$ ion is essential in the binding of GABA-$^{14}C$ to the mitochondria-like fraction of rat brain.

From the above results, it is suggested that the $Na^+$ ion may be essential for the action of GABA. Details of this work will be published elsewhere in Japanese.

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