EFFECT OF X537A ON THE RELEASE OF AMINO ACIDS IN RETINA

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SUMMARY

The effect of the Ca^{2+} ionophore X537A on the release of neurotransmitters in the chick retina has been studied. X537A induces the release of three postulated neurotransmitters (taurine, glycine and γ-aminobutyrate) and does not affect the release of leucine. The X537A induced release of the neurotransmitters is accompanied by an enhancement in the uptake of $^{45}Ca^{2+}$. As the net amount of Ca^{2+} in retina is not modified by X537A, it is probable that the release of neurotransmitters results from an enhancement in the turnover of Ca^{2+} across the membrane. The relative quantitative release of the three neurotransmitters is modified by the presence of Na^{+}.

It has been known for a number of years that in neural tissue a depolarizing current induces the release of neurotransmitters provided Ca^{2+} is present in the incubation mixture (1,2). As either the ommission of Ca^{2+} or the addition of ethylenediaminetetraacetate to the system (3) prevents the release of neurotransmitters, it is generally accepted that Ca^{2+} is absolutely required in the phenomenon. With respect to its mechanism of action, Miledi and Slater (4) observed that the iontophoretic application of Ca^{2+} to presynaptic terminals failed to induce the release of neurotransmitters and thus, they suggested that the interaction of Ca^{2+} with a membrane component was required for neurotransmitter release. Nevertheless it is not known whether in the absence of a depolarizing current the flux of Ca^{2+} through the membrane or the interaction of Ca^{2+} with a membrane component suffices to induce neurotransmitter release.

In this work an attempt to answer this question has been made

by measuring the effect of the Ca^{2+} ionophore X537A (5,6) on the release of neurotransmitters in the chick retina. The results show that in the absence of a depolarizing current, the translocation of Ca^{2+} as mediated by X537A induces the release of three amino acids that have been postulated to act as neurotransmitters in retina: taurine (7,8), glycine (9), and γ-aminobutyrate (10,11).

MATERIAL AND METHODS

The technique for measuring the release of neurotransmitters in retina has been described previously (7). The retinae from male, 3-5 weeks old, Sex Link chickens, adapted to the dark for 2 hr, were used in all the experiments. Excised retinae (about 40 mg wet weight) were incubated with shaking at 37°C for 15 min in 10 ml of oxygenated Krebs Ringer bicarbonate buffer that contained 5.1 mM glucose and either: ^{35}S-taurine (2.5 µC/ml), ^{14}C- γ-aminobutyrate (0.2 µC/ml), ^{14}C-glycine (0.5 µC/ml) or ^{14}C-leucine, (0.1 µC/ml). The retinae once loaded with a labeled amino acid were washed and transferred to fresh Krebs Ringer bicarbonate buffer. In some experiments the Na^{+} content of the buffer was replaced by an equivalent amount of choline. The release of the amino acids was followed by measuring the radioactivity of aliquots of the incubation mixture withdrawn at the time indicated in the Results section. Ca^{2+} influx was determined by measuring the amount of $^{45}Ca^{2+}$ taken up by the retina after incubation in mixtures identical to those in which the release of amino acids was measured. At various time intervals the retinae were withdrawn and extensively washed with cold Krebs Ringer for 5 min so as to eliminate $^{45}Ca^{2+}$ that had been adsorbed to the surface of the cells. The dry weight of the retinae was determined and thereafter the radioactivity was measured after digestion of the tissue with 0.7 ml of NCS reagent (Amersham, Searle Co., Ill.). The total amount of Ca^{2+} in retinae was measured in a deproteinized extract of the tissue by flame photometry.

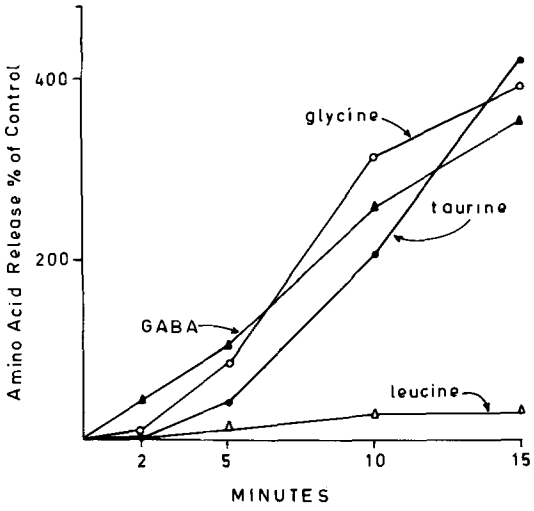

Fig. 1. Effect of X537A on the release of amino acids by chicken retinae. The release of amino acids was measured as described in Methods. The values shown indicate the percentage increase over the control in the release of the indicated amino acids induced by 13.3 µg per ml of X537A. About 10% of the labeled amino acid that was taken up during the loading conditions was released in the absence of X537A.

RESULTS

There are two ways through which neurotransmitter amino acids can be released in retina. One is the specific mechanism related to nerve transmission (12), and the other is via a less specific mechanism through which some amino acids, including the neurotransmitters, are ejected into the extracellular media (12). Figure 1 shows that X537A significantly enhances the release of three amino acids that have been postulated to act as neurotransmitters, taurine (7,8), glycine (9) and γ-aminobutyrate (10,11). As the release of leucine was not affected by the ionophore it is valid to conclude that X537A is acting on the mechanism specifically related to neurotransmission.

The ionophore X537A mediates the translocation of Ca^{2+} across a wide variety of membranes (5,6), however it is not absolutely specific for Ca^{2+}, as it may also facilitate the movement of Na^{+} (5). Indeed in the conditions of Figure 1, it was found that the content of Na^{+} in the

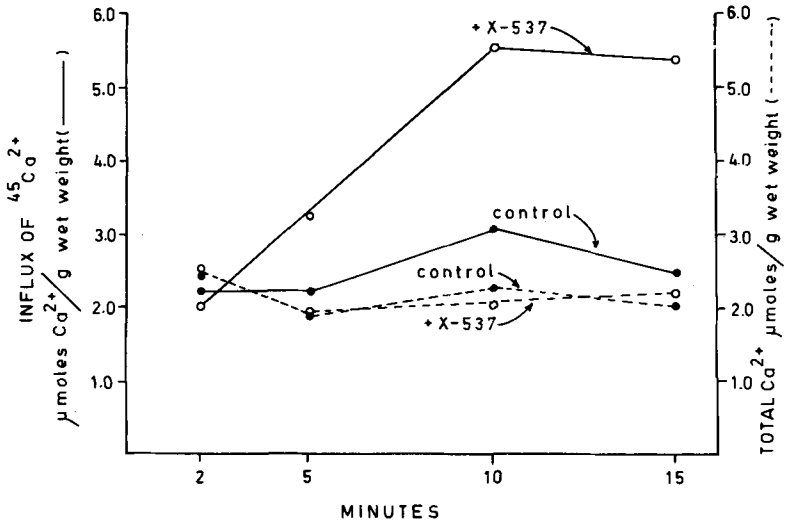

Fig. 2. Effect of X537A on the uptake of $^{45}Ca^{2+}$ and on the Ca^{2+} concentration of retinae. Retinae were incubated in Krebs-Ringer bicarbonate buffer (2.5 mM $CaCl_2$) in which Na^+ was omitted and substituted by an equivalent amount of choline. X537A was included where shown at a concentration of 13.3 μg per ml. At the indicated times a retina was withdrawn and either its content of $^{45}C^{2+}$ on its total Ca^{2+} content was measured.

retinae increased in a 15 minute period from a value of 79 nmoles per mg of wet weight to a value of 132 nmoles. As neural tissue possesses Na^+ sensitive amino acid carriers (12) and as Na^+ induces the spontaneous release of amino acids, including the neurotransmitters (12), the subsequent experiments were carried out in Na^+-free media to ascertain whether the X537A induced release of neurotransmitters was primarily related to Ca^{2+} fluxes. In these studies Na^+ was substituted by an equivalent amount of choline.

Although choline inhibits the influx of Ca^{2+} in nervous tissue (13), the results of Figure 2 indicate that X537A significantly enhances the uptake of Ca^{2+} by retinae. In our experimental conditions, the X537A mediated $^{45}Ca^{2+}$ uptake was not instantaneous and a significant difference was appreciated only after 5 min of incubation, a plateau in the uptake of $^{45}Ca^{2+}$ being reached within 10 min of incubation. The net uptake of $^{45}Ca^{2+}$ at this time is about 25 nmoles per mg of dry weight.

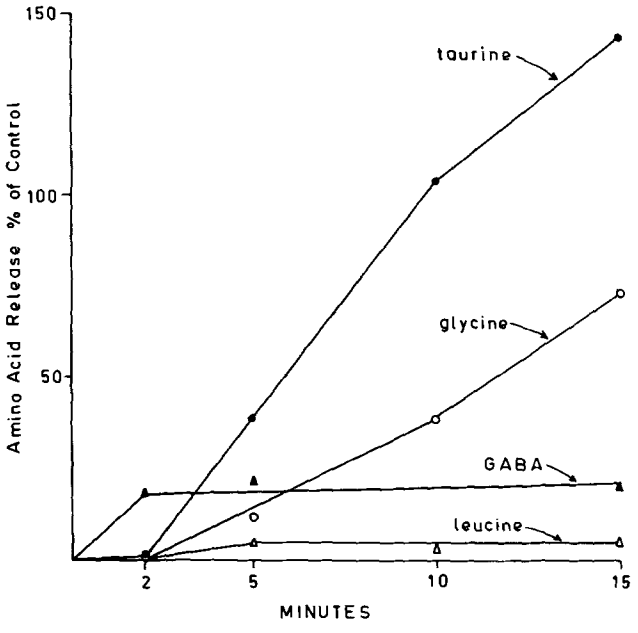

Fig. 3. Effect of X537A on the release of amino acids by chicken retinae incubated in the absence of Na^+. The experimental conditions were as in Fig. 1 except that the Na^+ content of the Krebs-Ringer bicarbonate buffer was substituted by an equivalent amount of choline.

In spite of the net gain of $^{45}Ca^{2+}$, the total amount of Ca^{2+} in retina was not modified by the ionophore, and the value of total Ca^{2+} remained constant for the length of the experimental period (Fig. 2). These findings are highly suggestive that in retina X537A merely enhances the turnover of Ca^{2+} across the membrane.

The effect of X537A on the release of taurine, glycine, γ-aminobutyrate and leucine by retinae incubated in a Na^+-free media is illustrated in Figure 3. The ionophore effectively elicited the release of the neurotransmitters and did not affect the release of leucine. Apparently the release of the three postulated neurotransmitters parallels the enhancement of the movement of Ca^{2+} across the membrane (compare Figures 2 and 3). However, if the results of Figures 1 and 3 are compared it may be observed that in the presence of Na^+, the release of glycine is similar to that of taurine, while in its absence the

release of taurine is more important than that of glycine. The release of γ-aminobutyrate is markedly enhanced in the presence of Na^+.

DISCUSSION

The results of this work indicate that the calcium ionophore X537A specifically induces the release of neurotransmitters. This release takes place in the absence of externally added Na^+ and parallels the movement of Ca^{2+} across the membrane. Therefore it may be concluded that an enhancement in the turnover of Ca^{2+} across the membrane elicits the release of neurotransmitters. Moreover the amount of taurine released by X537A is almost equal to that ejected after a light stimulus (see Fig. 1 in ref. 7).

This conclusion would, in a certain sense, be in agreement, but not necessarily prove the hypothesis of Miledi and Slater (4) that the interaction of Ca^{2+} with the membrane is the factor responsible for the release of neurotransmitters; most certainly a number of other factors are also involved. The finding that the release of neurotransmitters as mediated by X537A presents a different pattern in the presence or absence of Na^+ suggests that the process of neurotransmitter release is regulated by a complex system of ion movements. In this respect studies carried out on the effect of different ionophores on the release of various neurotransmitters may be of significant value in the understanding of the phenomenon.

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REFERENCES

1. Hubbard, J.I., Progr. Biophys. Molec. Biol. 21, 33
2. Katz, B. The Release of Neural Transmitter Substances, A. Thomas, Springfield, Ill., (1969).
3. Hammerstad, J.P., Murray, J.E. and Cutler, R.W.P., Brain Res. 35, 357 (1971).
4. Miledi, R. and Slater, C.R., J. Physiol. 184, 473 (1966).

5. Pressman, B.C., Fed. Proc. 82, 1698 (1973).
6. Scarpa, A. and Inesi, G. Fed. Eur. Biochem. Soc. Letters 22, 273 (1972).
7. Pasantes-Morales, H., Urban, P.F., Klethi, J. and Mandel, P. Brain Res. 51, 375 (1973).
8. Pasantes-Morales, H., Bonaventure, N., Wioland, N. and Mandel, P. Int. J. Neurosciences (1974) In press.
9. Bruun, A. and Ehinger, B. Invest. Ophthalmol. 11, 191 (1972.
10. Scholes, W.N. and Roberts, E., Biochem. Pharmacol. 13, 1319 (1964).
11. Graham, L.T., Baxter, C.F. and Lolley, R.N. Brain Res. 20, 379 (1970).
12. Hammerstad, J.P. and Cutler, R.W.P. Brain Res. 47, 401 (1972).
13. Diamond, I. and Goldberg, A.L. J. Neurochem 18, 1419 (1971).