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Prostaglandin release mediates drug-induced stimulation of sodium transport in frog skin: the effects of quinacrine

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1 Quinacrine markedly increased the release of prostaglandin E_2 (PGE_2) into the basolateral solution of the bullfrog skin from a control value of 32.7 ± 21.7 pg per 20 min period to a stimulated value of 8593.1 ± 4112.3 pg per 20 min period.

2 Quinacrine increased the amiloride-sensitive short circuit current from $20.7 \pm 2.1\ \mu A\ cm^{-2}$ to $45.4 \pm 6.5\ \mu A\ cm^{-2}$.

3 The stimulatory effects of quinacrine on both short circuit current and prostaglandin release were blocked in skins pretreated with indomethacin (10^{-6} M).

4 Quinacrine did not block either the stimulation of the short circuit current or the increase in PGE_2 release caused by the calcium ionophore, ionomycin.

5 These results suggest: (a) the release of PGE_2 and the stimulation of the short circuit current caused by quinacrine are linked since blocking PGE_2 release inhibits the stimulation of the short circuit current; (b) given the complexity of its actions, quinacrine is a poor tool to examine whether the effects of a given agent are mediated through the activation of endogenous phosopholipases. In addition our results taken together with other findings in the literature suggest that there is a diverse group of compounds that stimulate transepithelial sodium transport by releasing PGE_2.

Introduction

Quinacrine is an agent that can markedly inhibit the activity of phospholipase A_2 (Flower, 1978; Hofmann *et al.*, 1982). Based on this property, quinacrine has been used as a tool to determine whether or not the increase in prostaglandin E (PGE) release caused by a given procedure is mediated through the activation of endogenous phospholipases (Zusman *et al.*, 1977; Cuthbert & Wilson, 1981; Folkert & Schlondorff, 1983). The results described here were obtained when we attempted to use quinacrine in this manner to determine whether or not endogenous phospholipases are involved in the activation of prostaglandin release caused by calcium ionophores in the frog skin (Erlij *et al.*, 1981). This possibility was examined because, in other systems, Ca ionophores increase PGE by a mechanism that appears to involve activation of phospholipase A_2 (Pickett *et al.*, 1977).

When we found that contrary to our expectations, quinacrine stimulated prostaglandin release by the isolated skin of the frog, we decided to analyse further the effects of this drug.

Methods

The experiments were carried out on isolated abdominal skins of the bullfrog *Rana catesbeiana*. Paired pieces of skin dissected from the same animal were used to provide control and experimental tissues. Skins were mounted separating two hemichambers designed to insulate the damaged edges of the tissue from the bathing solutions (Erlij, 1976). The area of the skin exposed to the bathing solution was $4.2\ cm^2$. The volume in each half chamber was 10 ml.

In all the experiments the short circuit current (I_{sc}) was measured by clamping the transepithelial voltage continuously to 0 mV. To assess the transepithelial resistance we usually monitored the current deflections caused by changing the clamping potential to 10 mV for 500 ms every 30 s.

Prostaglandin determinations

PGE synthesis was estimated as PGE_2 released into the medium over consecutive 20 min periods. The 2 ml samples were immediately frozen and stored at −20°C

until the day of the assay. Samples assayed repeatedly at intervals as long as seven weeks produced the same results.

Prostaglandin content was measured in unextracted media by the radioimmunoassay method of Dray *et al.* (1975). Paired samples and control curves were always measured in the same assay. The addition of PGE_2 to medium that had been incubated with the skin yielded recoveries ranging between 93 and 98% (15, 30 and 60 pg in assays performed in three different experiments). The determinations were carried out with antisera purchased from the Institut Pasteur, Paris, France. Cross-reactivity (at $B/B_0 = 0.5$) for the PGE_2 antiserum was 4% with PGE_1 and less than 1% with the following compounds: thromboxane B_2, PGA_2, PGA_1 and PGF. These values are in good agreement with the manufacturer's specifications as well as with tests conducted by another laboratory on this antiserum (Forrest *et al.*, 1982).

Solutions

Ringer solution had the following composition (mmol l^{-1}) NaCl 115, KCl 2.5, $CaCl_2$ 1.8, Na_2HPO_4 2.16 and NaH_2PO_4 0.86. Quinacrine (mol. wt. 472.9) was obtained from ICN Pharmaceuticals Inc., K&K Labs Division. Quinacrine was dissolved in Ringer solution to make a 10^{-2} M stock solution. To obtain a final concentration of 10^{-4} M, 100 µl of the stock solution was added to the serosal bathing solution. The ionomycin used was a gift from the Squibb Institute for Medical Research; 20 mg of ionomycin (mol. wt. 709.9) was dissolved in 3 ml of ethanol and 10 µl of this stock solution was added to the 10 ml of Ringer solution bathing the serosal side of the epithelium. This made the final concentration 9.4 µM. Indomethacin (mol. wt. 357.8) was dissolved in ethanol to make a 1mM stock solution; 10 µl of this solution was then added to the serosal side of the epithelium to give the final concentration of 1 µM. Since indomethacin was dissolved in ethanol, 10 µl of ethanol were also added to the control epithelia.

Statistics

Results concerning groups of experiments are given as the mean ± s.e.mean. The one tailed Student's *t* test for paired variables was used to determine the level of significance. Values of $0.05 > P$ were considered significant.

Results

Figures 1 and 5 illustrate the effects of quinacrine on the release of PGE_2 into the basolateral solution. Release into the apical solutions is not described because, in agreement with previous observations (Erlij *et al.*, 1981), we found that prostaglandin release into the apical solution was negligible, both under control conditions and during the action of agents that markedly activate prostaglandin release into the basolateral solution. The spontaneous release into the basolateral solution was extremely low, on occasions being below the limit of detection of our technique. In the six experiments in which we measured the effects of quinacrine on PGE_2 release, the average release in the 20 min period before the addition of quinacrine was 32.7 ± 21.7 pg. The addition of quinacrine (10^{-4} M) to the basolateral solution produced a rapid and large increase in PGE_2 release that decayed rapidly with time. In the 20 min period immediately following the addition of quinacrine the release of PGE_2 reached its maximal value which averaged 8593 ± 4112 pg. The difference from the spontaneous rate is significant ($0.05 > P$). In the collection period starting 60 min after the addition of quinacrine, the release of PGE_2 had dropped to 493.5 ± 551.8 pg. This level is not significantly different ($0.5 > P$) from the spontaneous release.

Figure 2a illustrates one out of 18 experiments in which we examined the effects of quinacrine on the I_{sc} of the isolated frog skin. Addition of quinacrine resulted in a significant stimulation of I_{sc} ($0.005 > P$). It rose from 20.7 ± 2.1 to 45.4 ± 6.5 µA cm^{-2}. At the

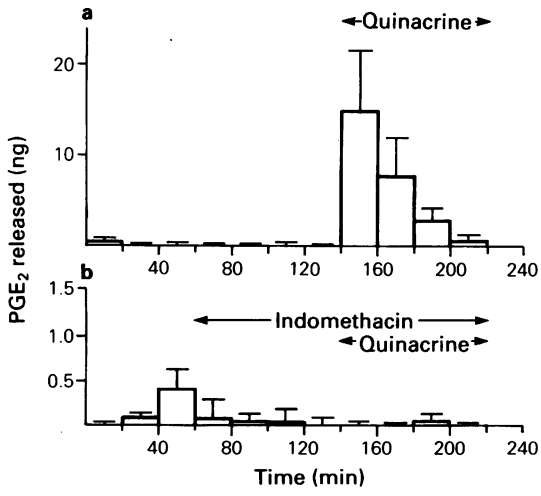

Figure 1 Effects of quinacarine on the release of prostaglandin E_2 (PGE_2) into the basolateral solution of the frog isolated skin. Both groups of tissues received quinacrine (10^{-4} M) 140 min after the start of sampling. The tissues in (b) were also exposed continuously to indomethacin (10^{-6} M) beginning 60 min after the start of sampling ($n = 3$). Only results obtained in paired tissues are shown. Ordinate scale: amount of PGE_2 released during each 20 min collecting period. Notice the different scales in (a) and (b).

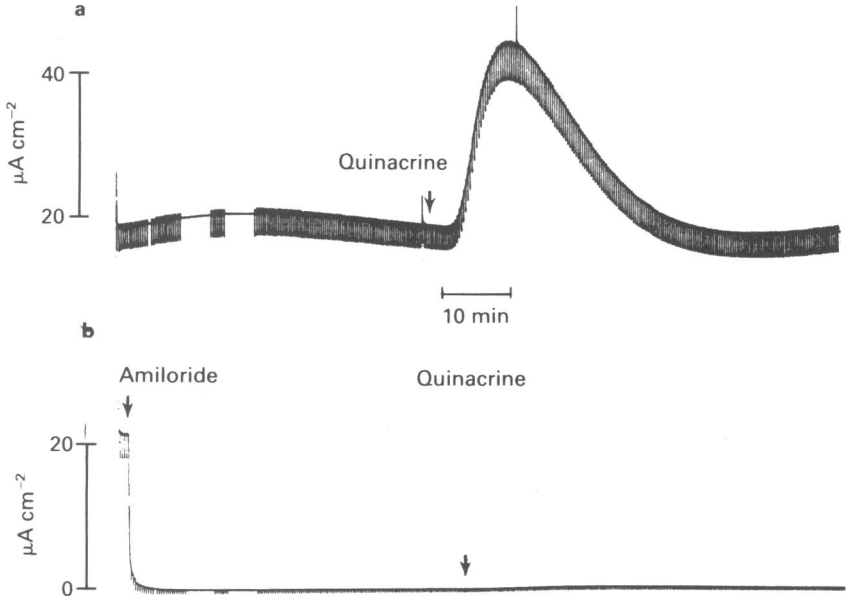

Figure 2 Effects of quinacrine on the I_{sc} of control and amiloride-treated skins. In (a) the tracing is recorded from the control portion of the skin. The tracing in (b) is from the paired tissue which first was treated with amiloride (10^{-4} M) in the apical solution and then received quinacrine (10^{-4} M) in the basolateral solution ($n = 6$). In this and the following figures we measured the transepithelial resistance by monitoring the current deflections caused by changing the clamping potential to 10 mV for 500 ms every 30 s. In addition to the deflections caused by the regular delivery of pulses, there are some larger sporadic vertical deflections. They were recorded when the short circuiting apparatus was briefly disconnected to read the transepithelial potential. The time calibration is the same for both tracings.

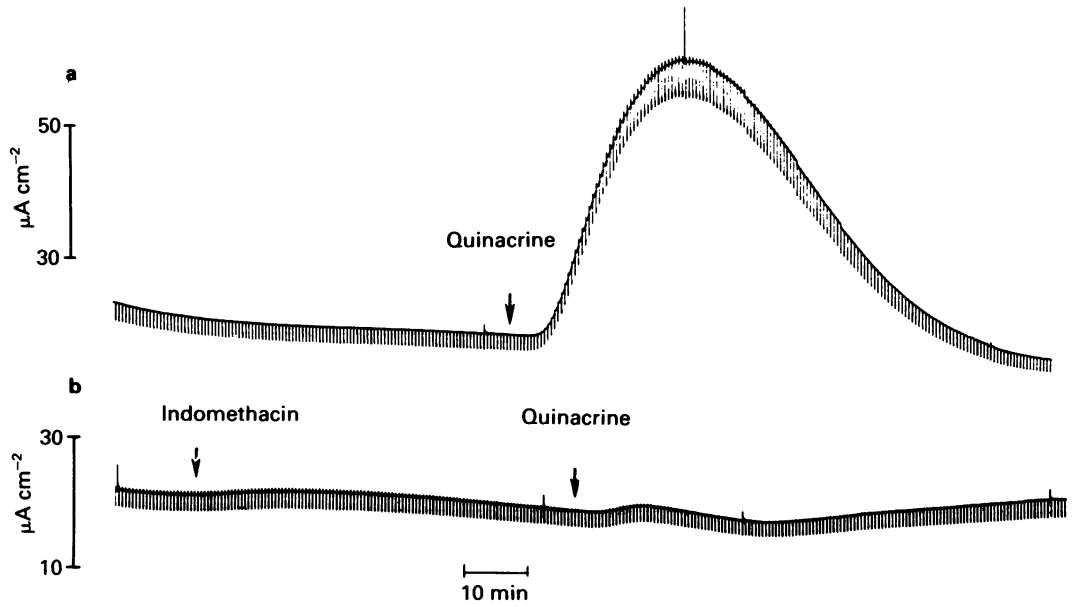

Figure 3 Effects of indomethacin on the quinacrine-induced stimulation of I_{sc} ($n = 5$). In (a) is shown the tracing recorded from the control portion of the skin. The tracing in (b) is from the paired tissue which first was treated with indomethacin (10^{-6} M) in the basolateral solution and then also received quinacrine in the basolateral solution. The time calibration is the same for both tracings.

peak of the stimulation the I_{sc} averaged 2.4 ± 0.3 times the resting level. The increase in I_{sc} decayed with time, returning to the resting level in about 45 min.

Nearly all the transepithelial Na current crosses the apical border of the epithelium through amiloride-sensitive channels (for reference, see Erlij & Ussing , 1978). To determine whether or not the increase in I_{sc} caused by quinacrine was due to activation of the amiloride-sensitive Na pathway we measured, in 6 experiments, the effects of amiloride on the quinacrine stimulation of I_{sc}. In Figure 2b are illustrated the results of adding quinacrine to the basolateral solution bathing a skin incubated with amiloride in the apical solution. The diuretic almost abolished the spontaneous I_{sc}. When quinacrine was given it significantly increased I_{sc} from 0.37 ± 0.08 to 0.59 ± 0.12 $\mu A\,cm^{-2}$ ($0.05 > P$).

We also examined whether the stimulation of both I_{sc} and PGE_2 release caused by quinacrine was modified by pretreatment with indomethacin. The results of these experiments are illustrated in Figures 1b and 3. Figure 1b shows that quinacrine did not increase PGE_2 release in indomethacin-treated tissues ($n = 3$). The effect of quinacrine on the I_{sc} of indomethacin-treated skins is shown in Figure 3. Figure 3a shows the characteristic stimulatory effects of quinacrine on the I_{sc} of the control portion of the skin. The paired test skin (Figure 3b) was incubated with indomethacin (10^{-6} M) in the basolateral solution, beginning 30 min before the addition of quinacrine. The subsequent addition of quinacrine to the basolateral solution was without effect on I_{sc}. In 5 control skins, the peak I_{sc} after adding quinacrine averaged 2.04 ± 0.51 times the resting level ($0.05 > P$), while in the paired indomethacin-treated skins the peak I_{sc} after quinacrine was 1.04 ± 0.03 times the resting level ($0.1 > P$).

To test whether or not quinacrine was an effective blocker of PGE_2 synthesis we measured the effects of quinacrine on the stimulation of PGE_2 release and I_{sc} caused by ionomycin. One of the six experiments carried out to examine the effects of quinacrine on the response to ionomycin is illustrated in Figure 4. The control skin (Figure 4a) was treated only with ionomycin. In agreement with previous observations (Erlij *et al.*, 1981) the addition of the ionophore produced a marked increase in I_{sc}. The paired experimental skin (Figure 4b) was first treated with the quinacrine which produced its usual stimulatory effect. When the quinacrine-induced stimulation had waned, ionomycin was added to the basolateral solution. The effects of the ionophore on I_{sc} were not

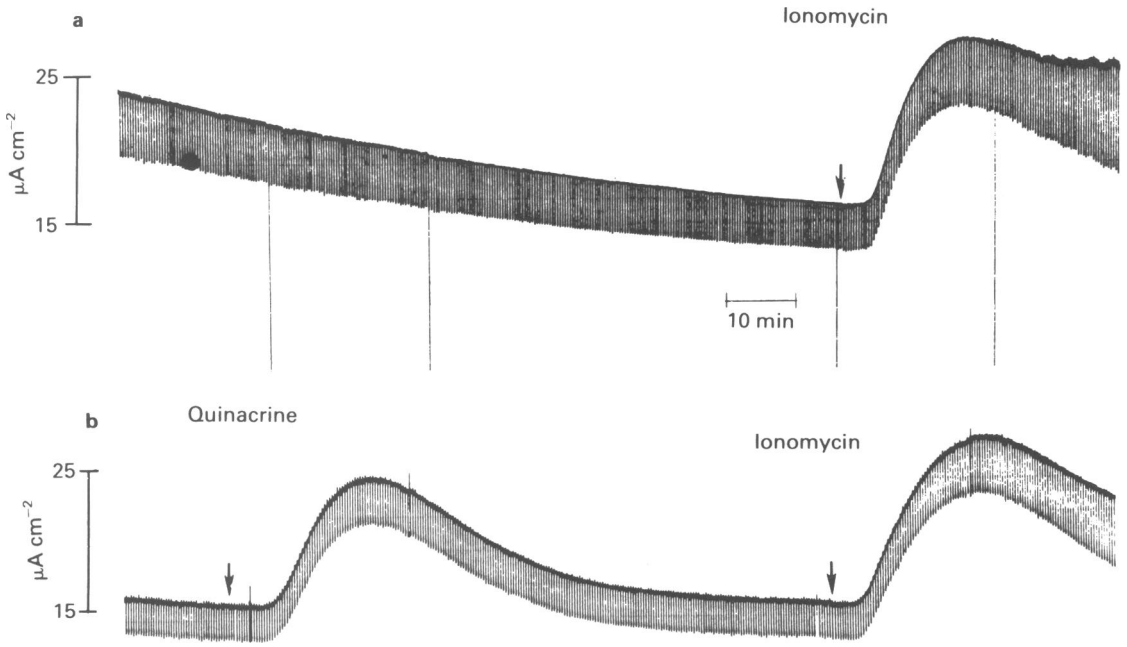

Figure 4 Effects of ionomycin on the short circuit current of quinacrine-treated skins. In (a) is shown the tracing obtained from the control portion of the skin that received ionomycin (9.4×10^{-6} M) only ($n = 6$). The tracing (b) is from the paired tissue which first received quinacrine and then was treated with ionomycin. Both substances were added to the basolateral solution. The time calibration is the same for both tracings.

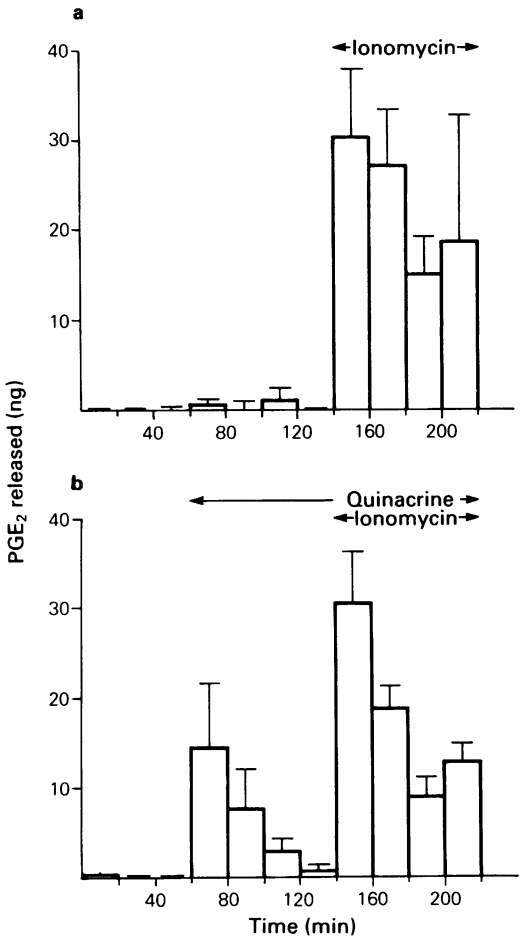

Figure 5 Effects of quinacrine on the release of prostaglandin E_2 (PGE_2) induced by ionomycin. (a) Shows the effects of ionomycin (9.4×10^{-6} M) in the control skins ($n = 3$); (b) shows the effects of ionomycin in the paired skins that had been pretreated with quinacrine. Ordinate scale: amount of PGE_2 released during each 20 min collecting period.

significantly different from those observed in tissues that had not been exposed to quinacrine ($0.1 > P$).

Figure 5 summarizes 3 experiments in which we examined whether or not quinacrine modified the stimulation of PGE_2 release caused by ionomycin. As in previous experiments (Figure 1) quinacrine produced a transient stimulation of PGE release that returned to the basal level with time. Sixty minutes after the addition of quinacrine the rate of release had returned to a value of 706.9 ± 719.7 pg per 20 min period. Addition of ionophore to these quinacrine-treated skins increased PGE_2 release to 30542 ± 5751 pg during the first 20 min of treatment. This increase in the rate of PGE_2 release was not significantly different from that caused by ionomycin in the control tissues where the rate increased from 29.6 ± 59.2 pg per 20 min period to a value of 30515 ± 7517 pg during the 20 min period immediately following the addition of the ionophore ($0.5 > P$).

Discussion

The present experiments show that quinacrine increases both prostaglandin E_2 release and I_{sc} in the isolated skin of the frog. Since it is also well known that PGE_2 is a powerful activator of I_{sc} (Fassina *et al.*, 1969; Lote *et al.*, 1974; Gerencser, 1978; Els & Helman, 1981), it is conceivable that the activation of I_{sc} caused by quinacrine is due to the release of PGE_2 from the isolated skin. In agreement with this suggestion is the finding that indomethacin blocked the quinacrine-induced stimulation of both I_{sc} and PGE_2 release.

There is a point related to the above suggestion that requires additional comment. The maximal release of PGE_2 observed after the addition of quinacrine was lower than that reached with ionomycin (Figure 5). Nevertheless, both agents produced quite similar activations of I_{sc} (Figure 4). One interpretation is that ionomycin induced the release of so much PGE_2 that concentrations in the vicinity of the receptor were many times those required for a maximal stimulation of I_{sc}. The highest concentration of PGE_2 that we measured in the basolateral solution during the action of quinacrine was $1.45\ ng\ ml^{-1}$, whereas during the action of ionomycin, PGE_2 concentration reached $3.05\ ng\ ml^{-1}$. Both concentrations are below the concentration of around $100\ ng\ ml^{-1}$ needed to produce a maximum stimulation of I_{sc} when exogenous PGE_2 is added to the basolateral solution (Erlij & Gersten, unpublished observations). Since PGE_2 is initially released into the small intercellular space that surrounds the epithelial cells, it is not difficult to imagine that concentrations near the prostaglandin receptors were many times higher than those attained in the 10 ml of incubation solution from which we sampled. Indeed, measurements with inulin gave a value of $0.783\ \mu l\ cm^{-2}$ for the extracellular space of the isolated epithelium of the frog skin (Aceves & Erlij, 1971). Since we have $4.2\ cm^2$ of skin in our chamber the extracellular volume of the epithelium is about 3200 times smaller than the volume of incubation fluid used in our experiments.

Another point that deserves comment emerges when our findings are taken together with other authors' observations. It appears that there is a growing list of agents that produce an indomethacin-sensitive stimulation of I_{sc}. In additon to quinacrine,

the list so far includes angiotensin II, acetylcholine and other muscarinic agonists (Cuthbert & Wilson, 1981) and the Ca ionophores ionomycin and A23187 (Erlij *et al.*, 1981). Evidently, the stimulation of I_{sc} caused by a relatively large group of substances may be mediated by the release of an intermediary rather than by direct interaction with the Na transport system. Thus, it is not necessary to postulate the existence in the transport system of either a modifier site that can interact with a group of substances of very different chemical structures or a variety of sites that can react each with one or more of the substances in the group.

Our experiments also have bearing on a methodological point, namely, the use of quinacrine as a selective inhibitor of phospholipase A_2 in studies that attempt to elucidate the role of endogenous phospholipases in the control of cellular processes. In the frog skin, Ca^{2+} ionophores stimulate both I_{sc} (Nielsen, 1978; Balaban & Mandel, 1979; Erlij *et al.*, 1981) and PGE_2 release (Erlij *et al.*, 1981). It has been suggested that the stimulation of I_{sc} is due to the enhanced release of PGE_2 (Erlij *et al.*, 1981). From observations in other systems it was reasonable to expect that the increase in PGE_2 release caused in the frog skin by the ionophores may be mediated by activation of phospholipase A_2 (Pickett *et al.*, 1977; Oelz *et al.*, 1978; Folkert & Schlondorff, 1983). However, when we used quinacrine to examine this possibility the drug proved of little use. First, quinacrine had a marked stimulatory effect on the synthesis of PGE_2 and, second, it failed to block the stimulation of PGE_2 release caused by ionomycin. Our experiments may not constitute a unique instance in which quinacrine has a complex action. Dise *et al.* (1982) have shown that there are direct interactions between quinacrine and membrane phospholipids. This finding suggests that the major effects of quinacrine may be due to a modification of membrane architecture rather than to the specific inhibition of a particular enzyme.

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