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STIMULATION BY INJECTED GUANOSINE TRIPHOSPHATE OF THE SODIUM EFFLUX IN BARNACLE MUSCLE FIBRES

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SUMMARY

1. A study has been made of the mechanism by which injected disodium GTP stimulates the ouabain-insensitive Na efflux in single muscle fibres from the barnacle, *Balanus nubilus*.

2. Injection of $GTPNa_2$ causes a stimulatory response which is usually transitory and almost completely reversed by injecting $MgCl_2$ (but not KCl).

3. Injected 5′-guanylylimidodiphosphate, Gpp(NH)p, mimics this action of GTP but the reversal seen with injected Mg^{2+} is less pronounced.

4. (i) Pre-treatment of these fibres with verapamil reduces the size of the stimulatory response to GTP and Gpp(NH)p. (ii) Pre-injection of protein kinase inhibitor (PKI) or regulatory subunits reduces the response as well. (iii) Pre-treatment with imipramine or trifluoperazine reduces the response to injected GTP; in combination with verapamil, a greater reduction in response is seen.

5. Injection of EDTA leads to a stimulatory response which is transitory. This response is largely abolished by verapamil.

6. Injection of cholera toxin leads to a sustained stimulatory rather than a transitory response. GTP or Gpp(NH)p when injected following peak stimulation by cholera toxin leads to a moderate sustained stimulation.

7. These results support the view that the stimulatory response to injected $GTPNa_2$ is the result of activation of Ca^{2+} channels and of increased availability of GTPMg and that these two conditions bring about activation of adenylate cyclase and hence activation of cyclic AMP-protein kinase by newly formed cyclic AMP.

INTRODUCTION

Injection of disodium GTP into single barnacle muscle fibres causes a transitory stimulation of the ouabain-insensitive Na efflux (Bittar, Schultz & Harkness, 1977). The connexion between GTP and the behaviour of the Na efflux is of interest in several respects: first, GTP is regarded as being the source of energy for protein synthesis; secondly, GTP is the specific substrate of the guanylate cyclase system (Goldberg & Haddox, 1977); thirdly, GTP is an allosteric effector of the adenylate cyclase system (Bennett, Mong & Cuatrecasas, 1975). As will be shown in this paper, elucidation of the problem of how GTP acts has been considerably simplified by the use of several tools, notably a non-hydrolysable analogue of GTP. The evidence

brought forward is compatible with the view that the calmodulin-dependent form of adenylate cyclase plays a major role in the response to GTP and that activation of Ca^{2+}-channels is also an interrelated key factor.

METHODS

Specimens of *Balanus nubilus* were supplied by Mr David King at Friday Harbor, Seattle and kept in an Instant Ocean aquarium containing aerated artificial sea water (ASW). The temperature of the ASW was maintained at 12 °C. Single muscle fibres were isolated by dissection from the three pairs of depressor muscle bundles and cannulated in the same way as crab muscle fibres (Caldwell & Walster, 1963). A 50–80 mg weight was attached to the tendon end of the cannulated fibre. The composition of the ASW used as bathing medium was as follows (mM): NaCl, 465; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 10; $NaHCO_3$, 10; pH 7·8. Adjustment of the pH of ASW was carried out by adding HCl or NaOH.

The micro-injector

The micro-injector used was of the type described by Bittar & Tallitsch (1975). The volume of test fluid ejected was in the region of 0·3–0·4 μl. Since the fibres employed in the present experiments had an intrafibre fluid volume of $\sim$ 40 μl., the dilution factor may be taken as 100-fold.

Radiosodium measurements

The method adopted for counting ^{22}Na in the effluent and fibre was that described by Bittar (1966) and Bittar, Caldwell & Lowe (1967). Counting was by means of a Beckman automatic γ-counter. The results obtained were treated in two ways: (i) the net efflux in ct/min per min was plotted against time on semilog paper, and (ii) the fractional rate constant for ^{22}Na efflux was plotted against time on linear paper. The fractional rate constant k is described by the expression:

$$k(\text{time}^{-1}) = \frac{\text{Efflux}}{\text{fibre count during collection period}}$$

Changes in the magnitude of the Na efflux were estimated on the basis of the fractional rate constant plot, as described by Bittar, Hift, Huddart & Tong (1974). The results are given here as means $\pm$S.E. of means. Significance levels were computed by Student's t test. All experiments were done at 22–25 °C.

Membrane potential measurements

This was essentially as described by Bittar & Sharp (1979).

Agents

Ouabain, EDTA and disodium GTP were purchased from Sigma Chemical Company, St Louis, MO. 5′-Guanylylimidodiphosphate sodium was purchased from P-L Biochemicals Inc., Milwaukee, WI. Verapamil HCl was obtained from Knoll Pharmaceutical Company, Whippany, NJ, imipramine HCl and trifluoperazine dihydrochloride salt from Smith, Kline & French Laboratories, Philadelphia, PA. Pimozide (R6,238) was obtained from Janssen Pharmaceutica Inc., New Brunswick, NJ. Cholera toxin (lots C2-2925 and E2-3439) was supplied by Schwartz/Mann, Orangeburg, NY. Both lots, when reconstituted to 0·1 ml. with water, contained 0·5 M-TRIS, 0·01 M-$EDTANa_2$, 0·03 M-NaN_3 and 2 M-NaCl, pH 7·5. Pure protein kinase inhibitor (PKI) was supplied by Dr E. Fischer of the Department of Biochemistry, University of Washington, Seattle. He also provided a sample of bovine heart cyclic AMP-protein kinase isozyme II regulatory subunits (R_2cAMP_4). This was in 50 % glycerol containing 30 mM-KH_2PO_4, 0·1 mM-EDTA and 1 mM-dithiothreitol. The material was dialyzed against 3 mM-HEPES in water, pH 7·2 in the cold room and dessicated by applying Sephadex G-10 to the dialysis bag.

RESULTS

Stimulation by injected $GTPNa_2$ and its reversal by Mg^{2+}

The observation that injected 0·5 M-$GTPNa_2$ causes a rise in the Na efflux in fibres pre-treated with 10^{-4} M-ouabain and that the effect is usually transitory has been confirmed. Summarized in Fig. 1 is the concentration–response curve for the stimulatory action of injected GTP on the ouabain-insensitive Na efflux. Although the extent of activation by GTP in low concentration is significantly different from zero, only the injection of 0·25 M- and 0·5 M-$GTPNa_2$ solutions causes a marked response. Taking into account 100-fold dilution by the myoplasm, the effective concentration would seem to fall in the 2–5 mM range. Since injection of $ATPNa_2$ but not ATPMg (in equimolar concentrations) mimics the response to GTP (see Bittar & Tong, 1975; Schultz & Bittar, 1978) and since the stability constant of GTP for

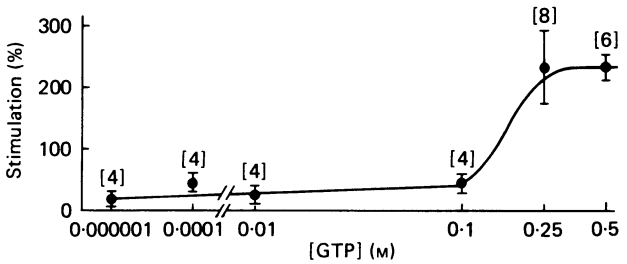

Fig. 1. Concentration–response curve for the stimulatory action of injected GTP on the ouabain-insensitive Na efflux. Vertical bars indicate ± S.E. of mean. The number of measurements carried out is given in parentheses. In experiments of this type where substances are injected, dilution by the myoplasm is roughly 100-fold.

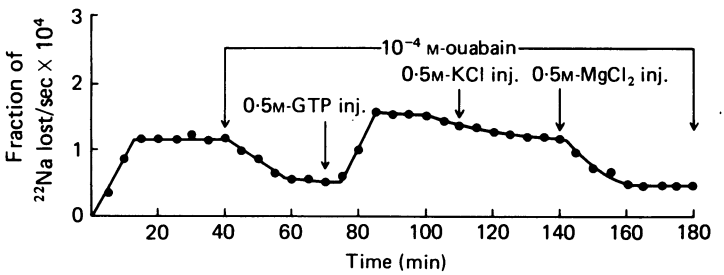

Fig. 2. Complete reversal of the stimulatory response to injected GTP by injecting $MgCl_2$. Also shown is that injection of KCl following onset of peak stimulation is without effect.

Mg^{2+} is practically the same as that of ATP for Mg^{2+} (Tu & Heller, 1974), it was deduced that GTP acts by removing internal free Mg^{2+}. Experiments therefore were carried out in which 0·5 M-$MgCl_2$ was injected following peak response to the injection of 0·5 M-$GTPNa_2$. The results show almost complete reversal of the stimulatory response ($n = 4$). Parallel experiments involving the injection of 0·5 M-KCl prior to $MgCl_2$ were also done ($n = 3$). As illustrated in Fig. 2, only injection of $MgCl_2$ is effective.

Stimulation by injected Gpp(NH)p and its reversal by Mg^{2+}

5′-Guanylylimidodiphosphate (Gpp(NH)p) is an analogue of GTP that is resistant to hydrolysis, e.g. by GTPase. However, unlike GTP, Gpp(NH)p reportedly activates adenylate cyclase after a lag phase (Londos, Salomon, Lin, Harwood, Schramm, Wolff & Rodbell, 1974; Rendell, Rodbell & Berman, 1977). It is shown in Fig. 3*A* that injection of 0·5 M-Gpp(NH)p causes a marked rise in the ouabain-insensitive Na efflux (in the order of 283±26%, $n = 11$), the onset of the effect being rather rapid. Also shown here is that injection of ~ 0·3 μl. H_2O following peak stimulation is without effect on the course of the stimulated efflux ($n = 8$). By contrast, injection of 0·5M-$MgCl_2$, as shown in Fig. 3*B*, leads to a partial reversal of the response (in the order to 76±4%, $n = 4$).

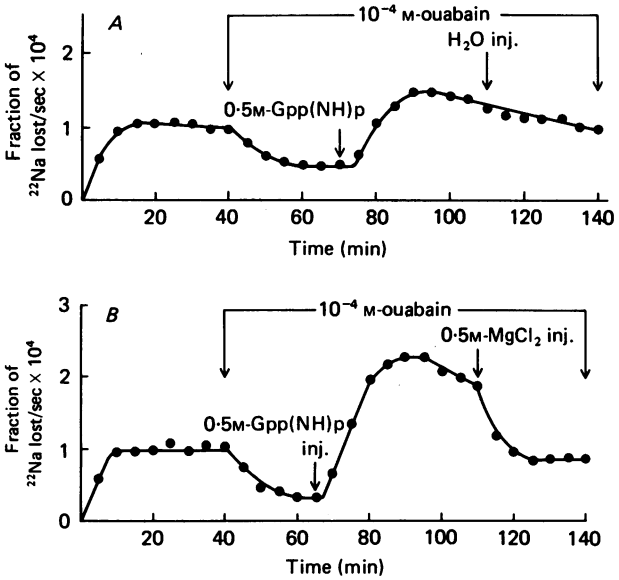

Fig. 3. *A*, the stimulatory response of the ouabain-insensitive Na efflux to injecting 0·5 M-Gpp(NH)p. Also shown is that injection of distilled water fails to influence the stimulated efflux. *B*, partial reversal of the stimulatory response to injected 0·5 M-Gpp(NH)p by injecting 0·5 M-$MgCl_2$.

Response to injected GTP and Gpp(NH)p in the presence of verapamil

There is evidence that GTP (Tsuda, Yatani & Goto, 1978) and Gpp(NH)p (Josephson & Sperelakis, 1978) are able to increase the slow inward current following external application to heart muscle. There also is evidence that the response of the ouabain-insensitive Na efflux to 100 mM-K ASW is completely abolished by pre-applying 10^{-4} M-verapamil (Mason-Sharp & Bittar, 1981). In view of these considerations, 10^{-4} M-verapamil was applied before injecting 0·5 M-GTP and 0·5 M-Gpp(NH)p. The results obtained with GTP show 149±9% stimulation ($n = 4$) *vs.* 267±35% in companion controls, $n = 4$ ($P < 0·025$), and with Gpp(NH)p 122±19% stimulation ($n = 4$) *vs.* 256±42% in companion controls, $n = 4$ ($P < 0·025$). This prompted trials with 10^{-3} M-verapamil but the fibres tolerated this high concentration poorly. Collectively, then, the results with verapamil were taken

to mean that the response to injected GTP and Gpp(NH)p is partly due to activation of Ca^{2+} channels. This conclusion is consistent with the observation that the response of the Na efflux to injected guanine nucleotide is dependent on external Ca^{2+} (Bittar & Nwoga, 1981).

Response to injected EDTA

The argument has been advanced that removal of internal free Mg^{2+} by injected GTP leads to activation of Ca^{2+} channels. One way of testing its validity is to inject EDTA and see whether the effect obtained not only mimics that of GTP but is also largely abolished by pre-applying verapamil. The results of experiments show that injection of 0·25 M-EDTA solution (at pH 7·0) causes a prompt and sharp rise in the ouabain-insensitive Na efflux which reaches a maximum within 15–20 min and then declines rather rapidly. The magnitude of the observed response averages $229 \pm 18\%$, ($n = 3$). In marked contrast, injection of EGTA fails to modify the behaviour of the ouabain-insensitive Na efflux (Bittar & Nwoga, 1981; Mason-Sharp & Bittar, 1981). If, however, 10^{-4} M-verapamil is applied externally prior to the injection of EDTA, the magnitude of the response is found to average $58 \pm 4\%$ ($n = 4$), a value which is significantly less than the preceding one (P being $< 0{\cdot}001$).

Membrane potential measurements

To check the possibility that activation of Ca^{2+} channels is the result of membrane depolarization, measurements were made of the resting membrane potential before and after injecting 0·5 M-$GTPNa_2$. Injection of $GTPNa_2$ into ouabain-poisoned fibres causes a fall in membrane potential (E_m) of $5{\cdot}6 \pm 0{\cdot}3$ mV ($n = 4$) or no change ($n = 2$) (fibres injected with 0·5 M-$GTPNa_2$ may or may not show a slight transitory contraction). A similar result has been demonstrated by the use of fibres pre-exposed to aldosterone (Bittar & Nwoga, 1981). Thus, the picture which emerges is that significant membrane depolarization is not an absolute requirement for Ca^{2+}-channel activation. That one can dispense with the need to depolarize the membrane is borne out by evidence coming from another type of experiment, e.g. fibres treated with halothane (Helmer, Rusy & Bittar, 1981). Halothane fails to reduce the membrane potential but stimulates the Na efflux mainly by a mechanism involving activation of Ca^{2+}-channels.

Response to GTP following injection of R_2cAMP_4

If the response to injected GTP is largely the result of newly formed cyclic AMP, and if cyclic AMP acts by activating cyclic AMP-dependent protein kinase (Bittar, Demaille, Fischer & Schultz, 1979), one would then expect injection of regulatory subunits to reduce the response to injected GTP. The results of experiments involving the injection of 10^{-5} M-R_2cAMP_4 30 min before injecting 0·5 M-$GTPNa_2$ show a marked reduction in the magnitude of the response to GTP ($103 \pm 21\%$ stimulation, $n, = 3$ *vs.* $277 \pm 10\%$ stimulation in companion controls, $n = 3$, $P < 0{\cdot}05$). It is perhaps noteworthy here that injected R_2cAMP_4 fails to modify the course of the ouabain-insensitive Na efflux.

Response to GTP following injection of protein kinase inhibitor (PKI)

The preceding experiments included a series where 10^{-5} M-PKI was injected into ouabain-poisoned fibres 30 min before the injection of 0·5 M-$GTPNa_2$. The results obtained show a stimulatory response to GTP averaging $107 \pm 22\%$, $n = 3$, compared with $277 \pm 10\%$, $n = 3$ ($P < 0{\cdot}05$).

Response to cholera toxin

Cholera toxin increases the basal activity of adenylate cyclase without altering the K_m for ATPMg (Gill, 1977). This activation results from inhibition of a GTPase lying in the regulatory subunit of the enzyme system (Cassel & Selinger, 1977*a*). It is illustrated in Fig. 4 that injection of 10^{-4} M-cholera toxin into a fibre pre-treated with

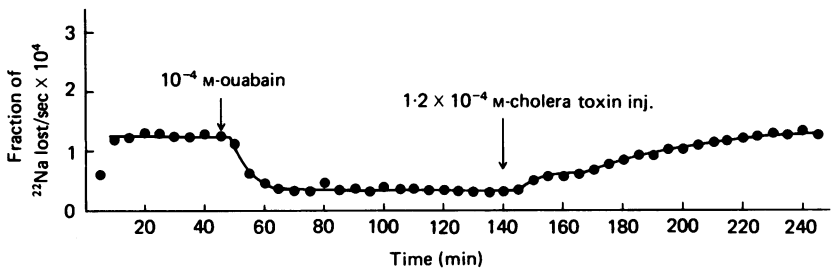

Fig. 4. The kinetic response of the ouabain-insensitive Na efflux to the injection of 10^{-4} M-cholera toxin.

10^{-4} M-ouabain causes a rather prompt but gradual rise in the efflux, which reaches a maximum within 80 min. The magnitude of this response averages $300 \pm 24\%$, $n = 11$. In sharp contrast, injection of the vehicle used to dissolve and preserve cholera toxin (see Methods) is without effect ($n = 5$). Characteristically, the response to cholera toxin is always sustained, a feature not seen following the injection of GTP or $CaCl_2$ (Schultz & Bittar, 1978) but seen following injection of catalytic subunit (Bittar *et al.* 1979) and GTP into fibres pre-exposed to aldosterone (Bittar & Nwoga, 1981). Cassel & Selinger (1977*a*,*b*) reported that Gpp(NH)p enhances the activation by cholera toxin of adenylate cyclase and that this analogue is more potent than GTP. Experiments therefore were done in which 0·5 M-GTP and 0·5 M-Gpp(NH)p were injected following the onset of peak stimulation by injected cholera toxin. The results indicate that the already highly stimulated Na efflux is still sensitive to these nucleotides and that the observed stimulatory response is sustained (Fig. 5). The combined responses to cholera toxin and GTP average $533 \pm 33\%$ ($n = 5$) as compared with $450 \pm 50\%$ stimulation in respect of cholera toxin and Gpp(NH)p together, $n = 5$, $P > 0{\cdot}25$. The response to cholera toxin alone averages $379 \pm 62\%$, $n = 5$, as observed in parallel experiments.

Response to GTP in the presence of imipramine, trifluoperazine and pimozide

The antidepressant drug, imipramine and several antipsychotic drugs such as trifluoperazine and pimozide are able to bind to calmodulin and hence inactivate the protein (Weiss & Levin, 1978). If this be true, and if calmodulin does modulate the

activity of the catalytic portion of adenylate cyclase (Wolff & Brostrom, 1979), then one would expect these drugs to reduce the response of the Na efflux to injected GTP. The results obtained show that fibres pre-treated with 10^{-4} M-ouabain and 5×10^{-5} M-imipramine are considerably less sensitive to injected GTP (0·5 M) than are control fibres (103 ± 28 % stimulation, $n = 5$ *vs.* 547 ± 53 % stimulation in controls, $n = 5$), P being $< 0{\cdot}001$. This is also the case with fibres pre-treated with 5×10^{-5} M-trifluoperazine and 10^{-6} M-pimozide, although the loss in sensitivity to GTP does not seem to be comparable to that seen following imipramine treatment (trifluoperazine: 214 ± 26 % stimulation, $n = 5$ *vs.* 547 ± 53 % stimulation in controls, $n = 5$, $P < 0{\cdot}005$; pimozide: 354 ± 29 % stimulation, $n = 5$ *vs.* 547 ± 53 % stimulation in controls, $n = 5$, $P < 0{\cdot}025$).

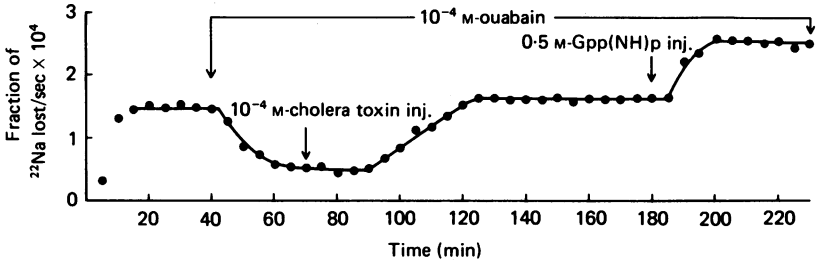

Fig. 5. Sustained stimulation by injecting 0·5 M-Gpp(NH)p following peak stimulation by cholera toxin.

The question now asked was whether application of verapamil together with imipramine (and verapamil together with trifluoperazine) would result in further reduction of the magnitude of the response of the ouabain-insensitive Na efflux to injected GTP. The results of these two groups of experiments are as follows: fibres pretreated wih 10^{-4} M-verapamil and 5×10^{-5} M-imipramine show 95 ± 7 % stimulation by injected GTP ($n = 4$) *vs.* 125 ± 10 % stimulation in controls, $n = 4$, i.e. fibres exposed to verapamil only ($P < 0{\cdot}05$). Fibres pre-treated with 10^{-4} M-verapamil and 5×10^{-5} M-trifluoperazine show 113 ± 16 % stimulation by injected GTP, $n = 4$ *vs.* 213 ± 12 % stimulation in controls, $n = 4$ ($P < 0{\cdot}005$). These results are significant for at least two reasons. One is that they provide evidence for the view that the calmodulin system stays active following inactivation of Ca^{2+} channels with verapamil. The other is the possibility that part of the response to GTP could be due to activation by internal free Ca^{2+} of Ca^{2+}-dependent protein kinase which is under the control of calmodulin. Evidence of a connexion between these two systems is available (Schulman & Greengard, 1978; Wolff & Brostrom, 1979).

DISCUSSION

The experiments described here, including those involving the injection of EDTA, provide evidence in favour of the view that GTP stimulates the ouabain-insensitive Na efflux as the result of removing internal free Mg^{2+}. Judging by the concentration–response curve, binding by GTP of Mg^{2+} seems stoichiometrical in the light of evidence that the internal free Mg^{2+} in barnacle fibres lies in the 3–5 mM range (Ashley & Ellory, 1972; Brinley, Scarpa & Tiffert, 1977). If the assumption that GTP binds

internal free Mg^{2+} is admitted, one is then justified in reasoning as follows. Removal of internal free Mg^{2+} by GTP (or Gpp(NH)p) leads to activation of Ca^{2+} channels in the absence of significant membrane depolarization. In turn, the consequent fall in myoplasmic pCa and increased availability of GTPMg bring about activation of adenylate cyclase and hence newly formed cyclic AMP. However, it cannot be supposed that activation by cyclic AMP of cyclic AMP-dependent protein kinase is the whole cause of the stimulatory response to injected GTP. This is because PKI and regulatory subunits only partially abolish the response. The fact that the response to GTP is fully reversed by injecting Mg^{2+} not only strengthens this interpretation but also provides a clue as to the nature of the second cause. Thus, for example, the effect of Mg^{2+} removal would be inhibition of phosphoprotein phosphatase, as observed in rabbit skeletal muscle (Kato, Kobayashi & Sato, 1975; Khatra & Soderling, 1977) and hence, phosphorylation would exceed dephosphorylation. Alternatively, GTP may remove internal trace elements such as Fe and Zn which are inhibitory to phosphoprotein phosphatase, e.g. in canine heart muscle (Li, Hsiao & Chan, 1978). Were this so, one would expect activation of phosphoprotein phosphatase to lead to an increase in the number of phosphate acceptor sites.

An explanation of why injected cholera toxin leads to a sustained stimulation rather than a transitory stimulation is unavailable. However, it is not without significance that this kinetic result resembles that seen following the injection of pure protein kinase catalytic subunits (Bittar, E. E., Chambers, G. & Fischer, E., unpublished). Whether the response to cholera toxin can be reversed by injecting Mg^{2+} or reduced by injecting PKI and regulatory subunits and whether a combination of verapamil and imipramine can practically abolish the response is not yet known.

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REFERENCES

ASHLEY, C. C. & ELLORY, J. C. (1972). The efflux of magnesium from single crustacean muscle fibres. *J. Physiol.* **226**, 653–674.

BENNETT, V., MONG, L. & CUATRECASAS, P. (1975). Mechanism of activation of adenylate cyclase by *Vibrio cholerae* enterotoxin. *J. Membrane Biol.* **24**, 107–129.

BITTAR, E. E. (1966). Effect of inhibitors and uncouplers on the Na pump of the *Maia* muscle fibre. *J. Physiol.* **187**, 81–103.

BITTAR, E. E., CALDWELL, P. C. & LOWE, A. G. (1967). The efflux of sodium from single crab muscle fibres. *J. mar. biol. Ass. U.K.* **47**, 709–721.

BITTAR, E. E., DEMAILLE, J., FISCHER, E. H. & SCHULTZ, R. (1979). Mode of stimulation by injection of cyclic AMP and external acidification of the sodium efflux in barnacle muscle fibres. *J. Physiol.* **296**, 277–289.

BITTAR, E. E., HIFT, H., HUDDART, H. & TONG, E. Y. (1974). The effect of caffeine on sodium transport, membrane potential, mechanical tension and ultrastructure in barnacle muscle fibres. *J. Physiol.* **242**, 1–34.

BITTAR, E. E. & NWOGA, J. (1981). Stimulation by injected GTP of the Na efflux in aldosterone pre-exposed barnacle muscle fibres. *J. Physiol.* **313**, 499–511.

BITTAR, E. E., SCHULTZ, R. & HARKNESS, C. (1977). Influence of insulin on sodium efflux in barnacle muscle fibers. *J. Membrane Biol.* **34**, 203–222.

BITTAR, E. E. & SHARP, D. M. (1979). Stimulaton by cyclic GMP of sodium efflux in barnacle muscle fibres. *J. Physiol.* **293**, 135–151.

BITTAR, E. E. & TALLITSCH, R. B. (1975). Stimulation by aldosterone of the sodium efflux in barnacle muscle fibres: effects of RNA inhibitors and spironolactone. *J. Physiol.* **250**, 331–346.

BITTAR, E. E. & TONG, E. (1975). Sensitivity of the sodium efflux in barnacle muscle fibers to the microinjection of ATP. *Life Sci. Oxford* **16**, 289–296.

BRINLEY, F. J., SCARPA, A. & TIFFERT, T. (1977). The concentration of ionized magnesium in barnacle muscle fibres. *J. Physiol.* **266**, 545–565.

CALDWELL, P. C. & WALSTER, G. E. (1963). Studies on the micro-injection of various substances into crab muscle fibres. *J. Physiol.* **169**, 353–372.

CASSEL, D. & SELINGER, Z. (1977*a*). Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site. *Proc. natn Acad. Sci. U.S.A.* **74**, 3307–3311.

CASSELL, D. & SELINGER, Z. (1977*b*). Catecholamine induced release of [^{3}H]Gpp(NH)p from turkey erythrocyte adenylate cyclase. *J. cyclic Nucl. Res.* **3**, 11–22.

GILL, D. M. (1977). Mechanism of action of cholera toxin. *Adv. cyclic Nucl. Res.* **8**, 85–118.

GOLDBERG, N. D. & HADDOX, M. K. (1977). Cyclic GMP metabolism and involvement in biological regulation. *A. Rev. Biochem.* **46**, 823–896.

HELMER, P., RUSY, B. & BITTAR, E. E. (1981). Sensitivity to halothane of the sodium efflux in single barnacle muscle fibers. *J. Pharmac. exp. Ther.* **217**, 248–253.

JOSEPHSON, I. & SPERELAKIS, N. (1978). 5′-guanylimido-diphosphate stimulation of slow calcium current in myocardial cells. *J. molec. & cell. Cardiol.* **10**, 1157–1166.

KATO, K., KOBAYASHI, M. & SATO, S. (1975). Inactivation and reactivation of phosphoprotein phosphatase of rabbit skeletal muscle. *J. Biochem.* **77**, 811–815.

KHATRA, B. S. & SODERLING, T. R. (1977). Effect of ATP and metals on rabbit skeletal muscle phosphoprotein phosphatase activity. *Fedn Proc.* **36**, 527.

LI, H-C., HSIAO, K-J. & CHAN, W. W. S. (1978). Purification and properties of phosphoprotein phosphateses with different substrate and divalent cation specificities from canine heart. *Eur. J. Biochem.* **84**, 215–225.

LONDOS, C., SALOMON, Y., LIN, M. C., HARWOOD, J. P., SCHRAMM, M., WOLFF, J. & RODBELL, M. (1974). 5′-guanylyl-imidodiphosphate: a potent activator of adenylate cyclase systems in eukaryotic cells. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3087–3090.

MASON-SHARP, D. & BITTAR, E. E. (1981). Stimulation by high external K of the sodium efflux in barnacle muscle fibers. *J. Membrane Biol.* **58**, 213–226.

RENDELL, M., RODBELL, M. & BERMAN, M. (1977). Activation of hepatic adenylate cyclase by guanyl nucleotides. *J. biol. Chem.* **252**, 7909–7912.

SCHULMAN, H. & GREENGARD, P. (1978). Ca^{2+}-dependent protein phosphorylation system in membranes from various tissues and its activation by calcium dependent regulator. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5432–5436.

SCHULTZ, R. & BITTAR, E. E. (1978). Studies of the mode of stimulation by external acidification and raising the internal free calcium concentration of the sodium efflux in barnacle muscle fibers. *Pflügers Arch.* **374**, 31–38.

TSUDA, Y., YATANI, A. & GOTO, M. (1978). Effects of exogenously applied guanosine triphosphate on membrane current and tension of bull frog atrial muscle. *J. molec. & cell. Cardiol.* **10**, 813–826.

TU, A. T. & HELLER, M. J. (1974). Structure and stability of metal–nucleoside phosphate complexes. In *Metal Ions in Biological Systems*, vol. 1, ed. SIGEL, H., pp. 1–49. New York: Marcel Dekker.

WEISS, B. & LEVIN, R. M. (1978). Mechanism for selectivity inhibiting the activation of cyclic nucleotide phosphodiesterase and adenylate cyclase by antipsychotic agents. *Adv. cyclic Nucl. Res.* **9**, 285–303.

WOLFF, D. J. & BROSTROM, C. O. (1979). Properties and functions of the calcium-dependent regulator protein. *Adv. cyclic Nucl. Res.* **11**, 27–88.