LONG-TERM POTENTIATION OF TRANSMITTER RELEASE INDUCED BY ADRENALINE IN BULL-FROG SYMPATHETIC GANGLIA

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SUMMARY

1. Long-term potentiation (l.t.p.) of transmitter release induced by adrenaline in bull-frog sympathetic ganglia was studied using intracellular recording techniques.

2. The quantal content of the fast excitatory post-synaptic potentials (fast e.p.s.p.s: evoked by the nicotinic action of acetylcholine) was potentiated for more than several hours after treatment with adrenaline (1–100 μM).

3. A similar l.t.p. of quantal content was produced consistently by isoprenaline (10 μM) and only in a certain fraction of cells by dopamine (10 μM). The l.t.p. induced by adrenaline (10 μM) was blocked by a β-antagonist, propranolol (1 μM), but not by an α-antagonist, phenoxybenzamine (1 μM).

4. Dibutyryl adenosine 3′,5′-phosphate (dibutyryl cyclic AMP) (0·8–1·0 mM), adenosine 3′,5′-phosphate (cyclic AMP) (4 mM), 3-isobutyl-1-methylxanthine (10 μM), caffeine (1–2 mM), and cholera toxin (2 μg ml^{-1}) applied for 20–30 min, all caused the l.t.p. of quantal content. By contrast, adenosine 5′-phosphate (AMP) (4 mM) and adenosine (4 mM) had no potentiating action.

5. Treatment of the ganglion with adrenaline (2·5–160 μM) or dibutyryl cyclic AMP (4 mM) for 15–30 min resulted in the l.t.p. of the frequency of miniature e.p.s.p.s.

6. The l.t.p. of quantal content induced by adrenaline was markedly suppressed by lowering temperature from 20–25 °C to 11–13 °C, and blocked by dibutyryl guanosine 3′,5′-phosphate (dibutyryl cyclic GMP) (100 μM) consistently when applied together, but inconsistently when given after adrenaline.

7. The post-synaptic sensitivity to acetylcholine was unchanged for at least 1 h after exposure to adrenaline (2·5–160 μM) or dibutyryl cyclic AMP (0·8–4 mM).

8. It can be concluded that adrenaline produces l.t.p. of transmitter release by activating a cyclic-AMP-dependent metabolic process through the activation of β-adrenoceptors, and that this mechanism is presumably regulated by a process involving endogenous guanosine 3′,5′-phosphate (cyclic GMP).

INTRODUCTION

Long-term potentiation (l.t.p.) of synaptic efficacy induced by neuronal activities in central neurones (Bliss & Lømo, 1973; Andersen, Sundberg, Sveen, Swann & Wigström, 1980; Baranyi & Fehér, 1981) has been suggested as a basis for a certain

aspect of learning and memory (cf. Bliss, 1979). Use-dependent types of l.t.p. were also found to exist in both mammalian (Dunant & Dolivo, 1968; Brown & McAfee, 1982, Briggs, Brown & McAfee, 1985a) and amphibian (Kumamoto & Kuba, 1983b; Koyano, Kuba, Kumamoto, Minota & Nohmi, 1983; Koyano, Kuba & Minota, 1985) peripheral ganglia, and both presynaptic (Koyano et al. 1985; Briggs, McAfee & McCaman, 1985b) and post-synaptic (Kumamoto & Kuba, 1983b; Koyano et al. 1985) mechanisms were suggested.

In addition to use-dependent l.t.p.s, another type of l.t.p. induced by a hetero-synaptic transmitter was found to exist in bull-frog sympathetic ganglia during a study on the inhibitory action of adrenaline (Kato, Koketsu, Kuba & Kumamoto, 1985). It was observed that the amplitude and quantal content of the fast excitatory post-synaptic potential (fast e.p.s.p.) were enhanced for a long time after a brief exposure to adrenaline or dibutyryl adenosine 3′,5′-phosphate (dibutyryl cyclic AMP). These findings appear to be important in three respects; (1) for understanding the mechanism of synaptic plasticity in general, (2) as the first evidence in vertebrata for heterosynaptic plasticity similar to the serotonin-induced potentiation in an *Aplysia* ganglion, the best-analysed model system for the mechanism of learning and memory (Kandel & Schwartz, 1982), and (3) in relation to the recent findings that monoamines play important roles in the l.t.p. in the vertebrate central nervous system (Bliss, Goddard & Riives, 1983).

At a preliminary stage of experiments (Kuba, Kato, Kumamoto, Koketsu & Hirai, 1981) the adrenaline-induced l.t.p. in bull-frog sympathetic ganglia was studied mainly with relatively high concentrations. Furthermore, its characteristics were described only in abbreviated form (Kuba et al. 1981; Kumamoto & Kuba, 1983a). For these reasons, we have investigated further the adrenaline-induced l.t.p. at relatively low concentrations and now report in detail its characteristics and the evidence for the possible involvements of various cyclic nucleotides and subsequent metabolic processes in the mechanism.

METHODS

B-type neurones of the ninth or tenth paravertebral sympathetic ganglion of the bull-frog (*Rana catesbeiana*) were studied (Nishi & Koketsu, 1960). The ordinary intracellular recording technique was employed. Composition of normal Ringer solution was (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; Na_2HPO_4, 1·3; and NaH_2PO_4, 0·7.

Micro-electrodes were filled with 3 M-KCl or 1 M-K_3 citrate (tip resistance, 30–100 MΩ). Penetration with an electrode filled with the latter-salt solution gave a larger resting potential of the ganglion cell. The fast e.p.s.p.s generated by the nicotinic action of acetylcholine (ACh: cf. Kuba & Koketsu, 1978) were recorded every 3 s in a low Ca^{2+}–high Mg^{2+} solution (Ca^{2+}, 0·7–0·9 mM; Mg^{2+}, 5·4–6·5 mM), whose tonicity was adjusted by altering Na^+ concentration. All the fast e.p.s.p.s were stored on FM tape (DC – 1 KHz) and their quantal content and size were later calculated from 100 or 200 fast e.p.s.p.s by both the variance and failure methods (del Castillo & Katz, 1954; see Koyano et al. 1985 for a detail of the method), using a microcomputer (DEC, MINC-11/03). In most experiments, quantal contents obtained by two methods were coincident as shown in Fig. 1 A and B (the better coincidence was obtained by the analysis of 200 fast e.p.s.p.s). Therefore, the average value obtained by the two methods was usually used. In several experiments, however, when the number of failures was less than 5 %, only the variance method was employed. When a fast e.p.s.p. was accompanied by an action potential during potentiation of transmitter release, the amplitude of this fast e.p.s.p. was assigned to be a value equivalent to the largest in the sampling period. When

the number of action potentials exceeded 5% of total in the sampling period. the data were discarded.

Since the phenomena to be studied lasted more than several hours, it was necessary to assess whether the parameters of the fast e.p.s.p. were constant under the control condition. Fig. 1*C* shows fairly good stability of the quantal content and size of the fast e.p.s.p.s recorded over 250 min in a control (drug-free) solution, although they fluctuated around a mean value. This constancy of the fast e.p.s.p.s was confirmed in three other cells.
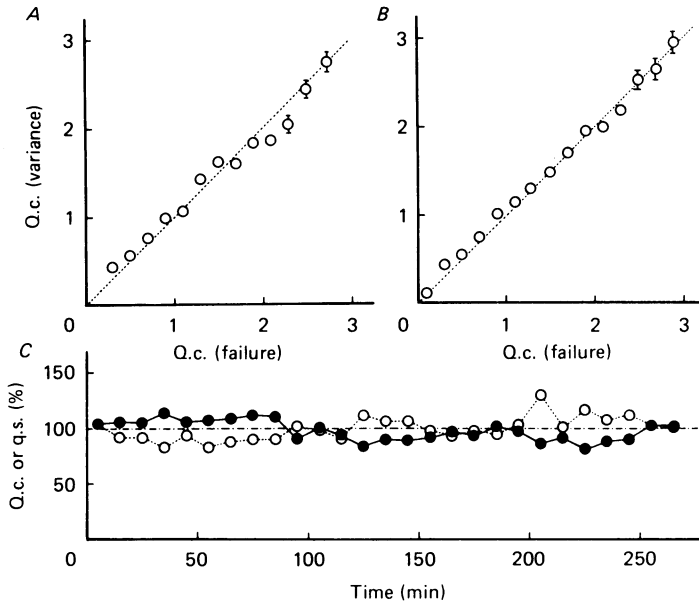

Fig. 1. *A* and *B*, correlation between the quantal content calculated by the variance method (q.c. (variance)) and that by the failure method (q.c. (failure)) from 100 (*A*) or 200 (*B*) fast e.p.s.p.s. Q.c.s (failure) were classified into groups, each having a range of 0·2 (0–0·20, 0·21–0·40, 0·41–0·60 and so on). The mean of q.c. (variance) and s.e. of mean were calculated from data of three to fifty-six sampling periods and plotted against each range of q.c. (failure). The s.e. of the values without a vertical bar was within the size of symbol. Correlation coefficients were 0·991 and 0·998 for *A* and *B*, respectively. *C*, quantal content (q.c.; ●) and size (q.s.; ○) of fast e.p.s.p.s recorded over 4 h in a low Ca^{2+}, high Mg^{2+} solution, showing their stability under the control condition. Each point was expressed as a percentage of the mean of all the values for each parameter.

ACh potentials were induced by ionophoretic application of ACh (2 M). They were judged to be generated by the nicotinic action of ACh, based on their fast time course. Miniature e.p.s.p.s (m.e.p.s.p.s) were recorded in a high K^+ (10 mM) solution to raise the basal level of their frequency. All experiments were carried out at room temperature (20–25 °C) unless otherwise specified. The method for lowering the temperature of a perfusing solution was similar to that used in a previous experiment (Kuba, 1980). Data were analysed for statistical significance using a Student's *t* test. Adrenaline HCl, dopamine HCl, phenoxybenzamine, propranolol and cholera toxin were obtained from Sigma. isoprenaline bitartrate from Nakarai Chem. Ind. Japan, dibutyryl cyclic AMP, dibutyryl guanosine 3′,5′-phosphate (dibutyryl cyclic GMP), adenosine, adenosine 3′,5′-phosphate (cyclic AMP) and adenosine 5′-phosphate (AMP) from P.-L. Biochem. Inc., U.S.A., caffeine (anhydrous) from Wako Pure Chem., Japan, and 3-isobutyl-1-methylxanthine (IBMX) from Aldrich.

<center>RESULTS</center>

L.t.p. of transmitter release induced by adrenaline

Adrenaline produces biphasic effects on transmitter release in bull-frog sympathetic ganglia; inhibitory action during exposure (Kato et al. 1985) and long-lasting potentiation after removal.

Time course of adrenaline-induced l.t.p. When the ganglion was treated with adrenaline at a concentration of 10 μM for 30 min, the amplitude and quantal content of the fast e.p.s.p. were decreased (Fig. 2 A b, B), or in some cells unchanged or slightly increased. By contrast, after the ganglion was washed with an adrenaline-free solution, the amplitude and quantal content were consistently enhanced for more
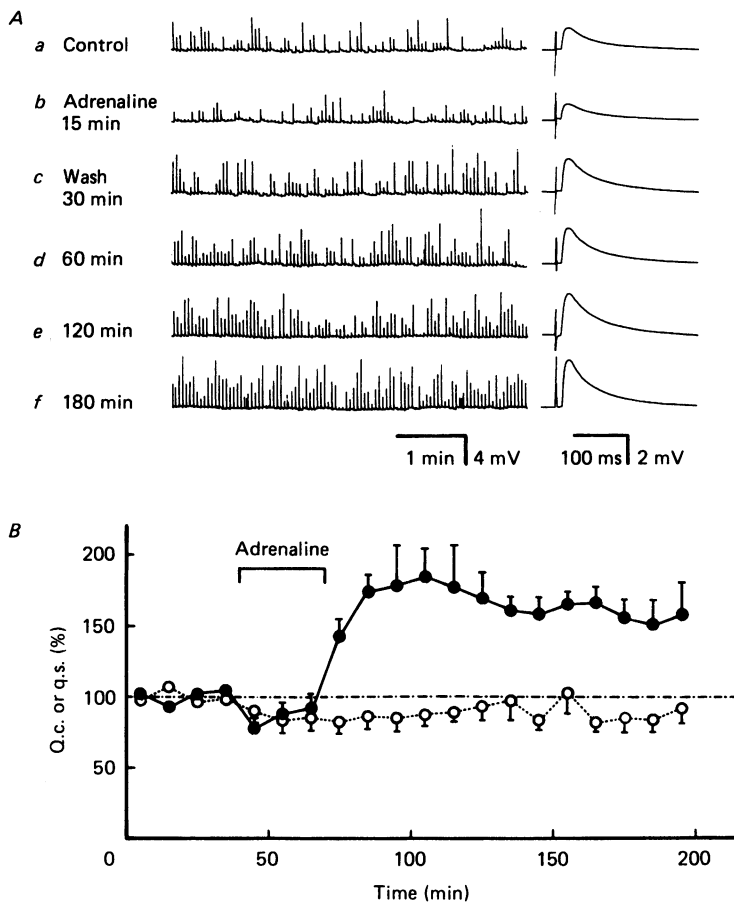

Fig. 2. Effects of adrenaline on the fast e.p.s.p.s. A, fast e.p.s.p.s before, during and after treatment with adrenaline (10 μM). Each trace shows fast e.p.s.p.s recorded on a pen-writing recorder (flat response up to 100 Hz) and their electronic average displayed in a fast time base. B, the time course of changes in quantal content (q.c.; ●) and quantal size (q.s.; ○) induced by application of adrenaline (10 μM). Each point is the mean and s.e. of mean of data obtained from five cells and expressed as a percentage relative to the control. The s.e. of the values without a vertical bar was within the size of symbol. Adrenaline was applied during the period indicated by a horizontal bar.

than several hours (Fig. 2 *A c–f*, *B*), indicating a l.t.p. of transmitter release. This adrenaline-induced l.t.p. remained unchanged in most cells (seven out of ten) for at least 2 h or dissipated very slowly over several tens of minutes in some (three) cells. A low concentration (1 μM) of adrenaline increased quantal content even during exposure without any sign of inhibition (Fig. 3 *A*), but with a slower rate of onset.

Dose–response curves for the adrenaline actions. Fig. 3 *A* shows dose–response curves for the actions of adrenaline on the quantal content of fast e.p.s.p.s during and after treatment. The facilitatory action appeared at a concentration less than 1 μM (adrenaline at 0·1 μM caused l.t.p.; Koketsu, Akasu, Miyagawa & Hirai, 1982 *a*), while the minimally effective concentration for the inhibitory action was greater than 1 μM. The magnitude of adrenaline-induced l.t.p. was maximal at concentrations greater than 10 μM. The duration of adrenaline-induced l.t.p. seems to be independent of adrenaline concentration.
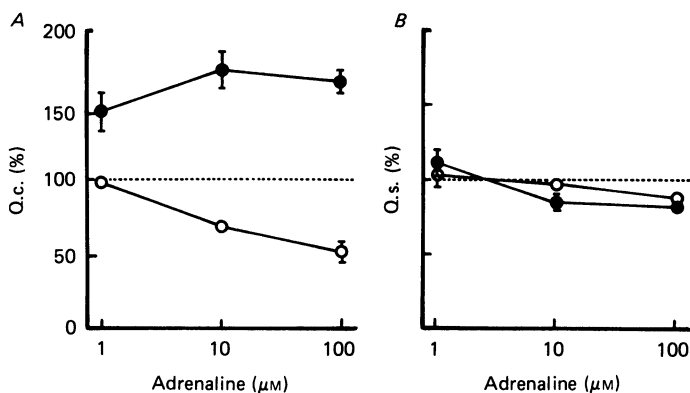

Fig. 3. Dose–response curves of adrenaline actions on quantal content (q.c.; *A*) and size (q.s.; *B*) during and after treatment. ○ represent the values during treatment with adrenaline for 20–30 min, while ● indicate the values 60 min after treatment. The s.e. of the values without a vertical bar was within the size of symbol. Data were obtained from four to fifteen cells.

The quantal size of the fast e.p.s.p. remained unchanged during and after the treatment with adrenaline (Fig. 3 *B*) except at 100 μM, where a small depression was observed (cf. Koketsu, Miyagawa & Akasu, 1982 *b*; Kato *et al.* 1985).

Effects on miniature excitatory post-synaptic potentials. Adrenaline (2·5–160 μM) produced a significant potentiation of the frequency of m.e.p.s.p.s during and after treatment (Fig. 4). In some cells, a decrease in frequency was observed in the presence of adrenaline (cf. Kato *et al.* 1985). However, the enhancement of frequency after wash was consistently seen, although the magnitude of the increase was variable among cells, and continued for at least several hours (Table 1). The mean amplitude of m.e.p.s.p.s was not changed significantly during and after treatment with adrenaline (Fig. 4 and Table 1).

No change in the ACh sensitivity of the post-synaptic membrane. The amplitude of ACh potentials induced by ionophoretic application of ACh remained unchanged for a long time after superfusion of the ganglion with adrenaline (10 μM; Fig. 5: 101 ± 9 %,
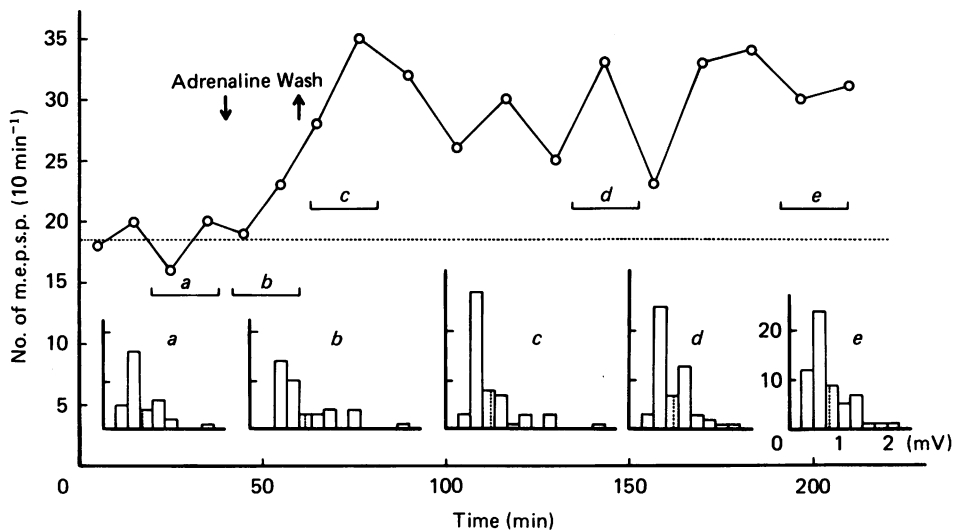
Fig. 4. Effects of adrenaline on m.e.p.s.p.s. Changes in m.e.p.s.p. frequency before, during and after application of adrenaline (5 μM) were plotted against time. Inset histograms are frequency distributions of the amplitude of m.e.p.s.p.s obtained from the period indicated by a horizontal bar with respective symbol below the time course of the m.e.p.s.p. frequency. Vertical dotted lines in each histogram indicate the mean amplitude. Adrenaline was applied during the time period indicated by downward and upward arrows.

TABLE 1. Effects of adrenaline (2·5–160 μM) on frequency and amplitude of m.e.p.s.p.s. Mean frequency (min⁻¹) and amplitude (mV) of control m.e.p.s.p.s are shown in parentheses

		Frequency 100 %	Amplitude 100 %
Control		(11·7 ± 6·0 (s.e.), n = 7)	(0·83 ± 0·22, n = 7)
Adrenaline			
During	0–20 min	158 ± 65 (n = 7)	108 ± 10 (n = 7)
Wash	10–30	266 ± 146 (n = 7)	106 ± 8 (n = 7)
	60–80	197 ± 39 (n = 6)	121 ± 9 (n = 6)
	100–120	146 ± 43 (n = 5)	109 ± 9 (n = 5)

$n = 5$, at 30–40 min after a 20 min exposure). This result, together with no change in the quantal size of the fast e.p.s.p. and the amplitude of m.e.p.s.p.s after wash-out of adrenaline, indicates that adrenaline-induced l.t.p. is solely presynaptic in origin.

The type of adrenoceptor for adrenaline-induced l.t.p.

L.t.p.s induced by dopamine and isoprenaline. Dopamine, which has α actions and was reported to produce a cyclic-AMP-dependent potentiation of slow e.p.s.p.s in mammalian ganglia (Libet, Kobayashi & Tanaka, 1975), showed variable effects on the quantal content of the fast e.p.s.p., although it consistently produced the inhibition during exposure (Kato *et al.* 1985). In four cells out of eight, there was no l.t.p. of quantal content (98, 108, 113 and 95 % of control at 30 min) after application of dopamine (10 μM; Fig. 6A). Four other cells exhibited a long-lasting increase in quantal content (287, 157, 127 and 145 %). In contrast, isoprenaline (10 μM), β-agonist, consistently brought about the l.t.p. of quantal content in all the cells
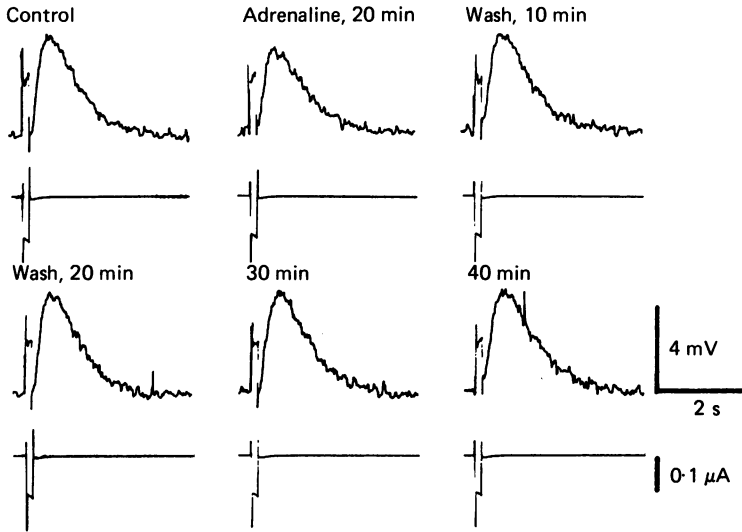
Fig. 5. Effects of adrenaline (10 μM) on ACh potentials induced by ionophoretic application of ACh. Duration of a current pulse for ionophoresis was 150 ms.

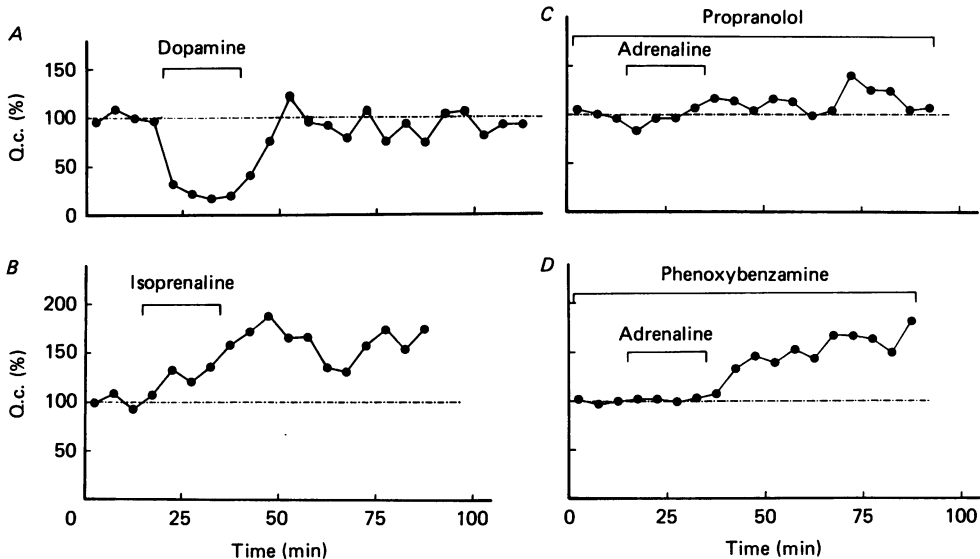Fig. 6. Experiments to determine the type of adrenoceptor involved in adrenaline-induced l.t.p. A and B, effects of dopamine (10 μM) and isoprenaline (10 μM) on quantal content (q.c.) of the fast e.p.s.p. during and after application of each of them. C and D, effects of adrenaline (10 μM) on quantal content in the presence of phenoxybenzamine (1 μM) or propranolol (1 μM). Data were expressed as a percentage of the control. Drugs were superfused to the ganglion during the time period indicated by a horizontal bar. Phenoxybenzamine (1 μM) and propranolol (1 μM) by themselves had no effect on quantal content ($108 \pm 6\%$, $n = 8$ and $107 \pm 6\%$, $n = 11$, of the control, respectively).

studied (Fig. 6B). Quantal content at 30 min after washing isoprenaline was 198 % (± 17 % of the control, $n = 5$). There was no significant change in quantal size throughout the l.t.p. induced by dopamine or isoprenaline.

Effects of adrenaline antagonists. In the presence of an α-antagonist, phenoxybenzamine (1 μM), adrenaline (10 μM) produced a significant potentiation of the quantal content of the fast e.p.s.p.s after a 20 min exposure (154 ± 9 % of the control, $n = 4$, significantly different from 100 % ($P < 0.02$), at 30 min after wash: Fig. 6D), which does not significantly differ from the adrenaline-induced l.t.p. produced in the absence of antagonist (162 ± 10 %, $n = 26$, significant from 100 % ($P < 0.001$), but not from that in the presence of antagonist ($P > 0.5$)). However, only a small or no l.t.p. was induced by adrenaline (10 μM) in the presence of a β-antagonist, propranolol (1 μM: 111 ± 6 %, $n = 6$, $P < 0.1$; 10 μM: 115 ± 18 %, $n = 4$, $P > 0.4$; Fig. 6C). These results and those in the foregoing section (except for the cases in which dopamine caused a l.t.p.) suggest that a β-adrenoceptor participates in the generation of adrenaline-induced l.t.p. Phenoxybenzamine at high concentrations (10 μM) reduced the magnitude of adrenaline-induced l.t.p. (quantal content: 120 ± 8 %, $n = 6$) (Kuba *et al.* 1981). This would presumably be due to a non-specific blocking action of this blocker on the presynaptic β-adrenoceptor.

Involvement of endogenous cyclic AMP and a metabolic process

Dibutyryl cyclic AMP and cyclic AMP mimic the potentiating actions of adrenaline. Dibutyryl cyclic AMP (0.8–1 mM) reduced slightly the amplitude of the fast e.p.s.p. (Fig. 7Ab; cf. Kato *et al.* (1985)) during the initial stage (10–20 min) of treatment with it. However, an exposure longer than 20 min to dibutyryl cyclic AMP caused an enhancement in the amplitude and quantal content of the fast e.p.s.p. (Fig. 7B). When the nucleotide was removed, there were further potentiations of the amplitude (Fig. 7Ac–f) and quantal content (Fig. 7B, filled circles), comparable in time course with those induced by adrenaline. Throughout potentiation, the quantal size of the fast e.p.s.p. remained essentially unchanged, although there was a small increase or decrease in some cells (Fig. 7B, open circles). Cyclic AMP (4 mM) also caused a similar, but smaller l.t.p. of quantal content (144 ± 10 % of the control, $n = 3$, $P < 0.05$, at 30 min) after a 20 min treatment without a change in quantal size (114 ± 5 %, $n = 3$, $P > 0.2$). The smaller effect of cyclic AMP could be due to a low permeability of the nucleotide to the cell membrane. The same explanation may be applicable to the lack of effect of cyclic AMP (4 mM) in a preliminary report (Kuba *et al.* 1981), where the nucleotide was applied only for 10 min.

The frequency of m.e.p.s.p.s increased for a long time after the treatment with dibutyryl cyclic AMP (4 mM) without a significant change in amplitude (Table 2).

AMP and adenosine fail to induce the l.t.p. Adenosine (4 mM) produced only a small, but insignificant enhancement of quantal content (115 ± 10 %, $n = 5$, $P > 0.1$, at 30 min) after a 20 min exposure without a significant change in quantal size (109 ± 5 %, $n = 5$). AMP (4 mM) did not show any potentiating actions (quantal content: 101 ± 12 %, $n = 6$) after treatment for 20 min, while it inhibited significantly both the quantal content (49 ± 10 %, $n = 6$, $P < 0.01$) and size (65 ± 11 %, $n = 6$, $P < 0.05$) during treatment.

Phosphodiesterase inhibitors induce l.t.p. When the ganglion was superfused with

A

a Control

b Dibutyryl
cyclic AMP
15 min

c Wash
30 min

d 60 min

e 120 min

f 180 min

.1 min | 4 mV   100 ms | 1 mV

B

Q.c. or q.s. (%)

Dibutyryl cyclic AMP

200

150

100

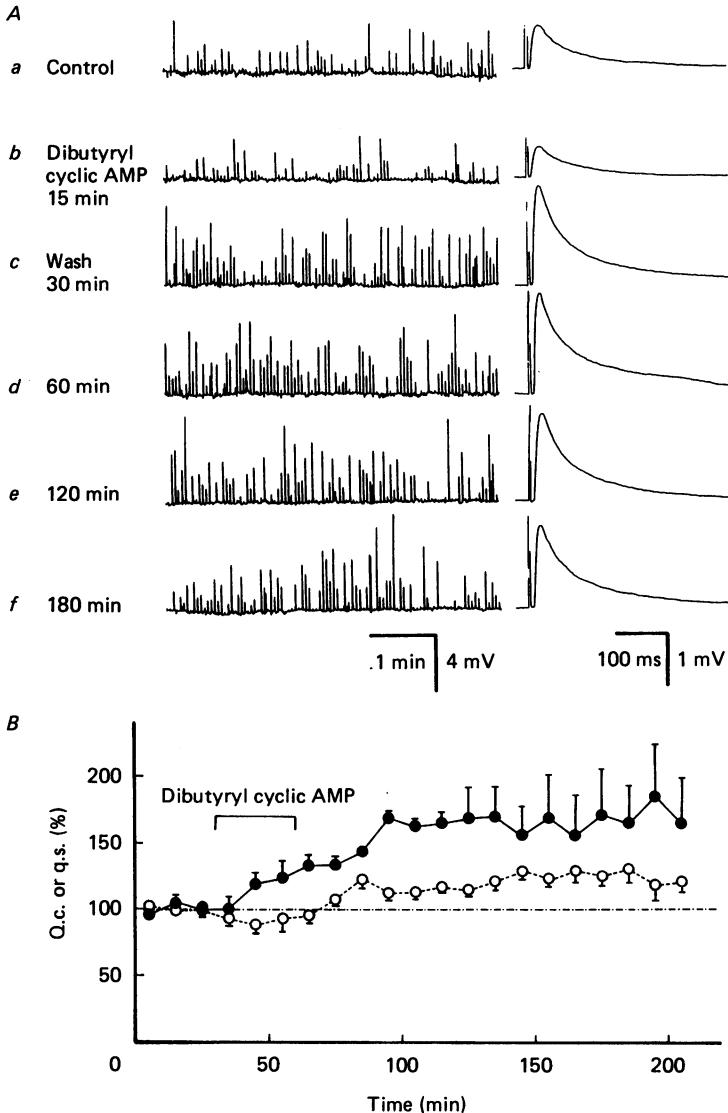50

0   50   100   150   200

Time (min)

Fig. 7. Effects of dibutyryl cyclic AMP on the fast e.p.s.p.s. *A*, fast e.p.s.p.s recorded before, during and after treatment with dibutyryl cyclic AMP (1 mM). Each trace shows the fast e.p.s.p.s recorded on a pen-writing recorder and their electronic average displayed in a fast time base. *B*, the time course of changes in quantal content (q.c.; ●) and size (q.s.; ○) before, during and after treatment with dibutyryl cyclic AMP (0·8–1 mM). Each point indicates the mean and s.E. of mean of data obtained from six different cells. Values were expressed as a percentage of the control. The s.E. of the values without a vertical bar was within the size of symbol. Nucleotide was applied during the time period marked by a horizontal bar.

TABLE 2. Effects of dibutyryl cyclic AMP (4 mM) on frequency and amplitude of m.e.p.s.p.s. Mean frequency (min^{-1}) and amplitude (mV) of control m.e.p.s.p.s are shown in parentheses

		Frequency 100%	Amplitude 100%
Control		$(13\cdot9\pm2\cdot5, n = 11)$	$(0\cdot87\pm0\cdot06, n = 11)$
Dibutyryl cyclic AMP			
During	5–10 min	$96\pm7\ (n = 10)$	$96\pm6\ (n = 10)$
Wash	5–10	$174\pm32\ (n = 10)$	$102\pm6\ (n = 10)$
	15–20	$172\pm31\ (n = 5)$	$105\pm9\ (n = 5)$
	45–50	$182\pm27\ (n = 4)$	$108\pm3\ (n = 4)$

a solution containing a phosphodiesterase inhibitor, IBMX (10 μM), for 20 min, the quantal content of the fast e.p.s.p. increased gradually or remained unchanged (Fig. 8A: the mean increase was significant with $P < 0\cdot01$ in Fig. 8Ba). However, the potentiation of quantal content occurred consistently after the removal of the drug (Fig. 8A, Ba; $P < 0\cdot05$). Caffeine, another phosphodiesterase inhibitor, also caused a similar potentiation of quantal content ($P < 0\cdot02$) after a 20 min exposure
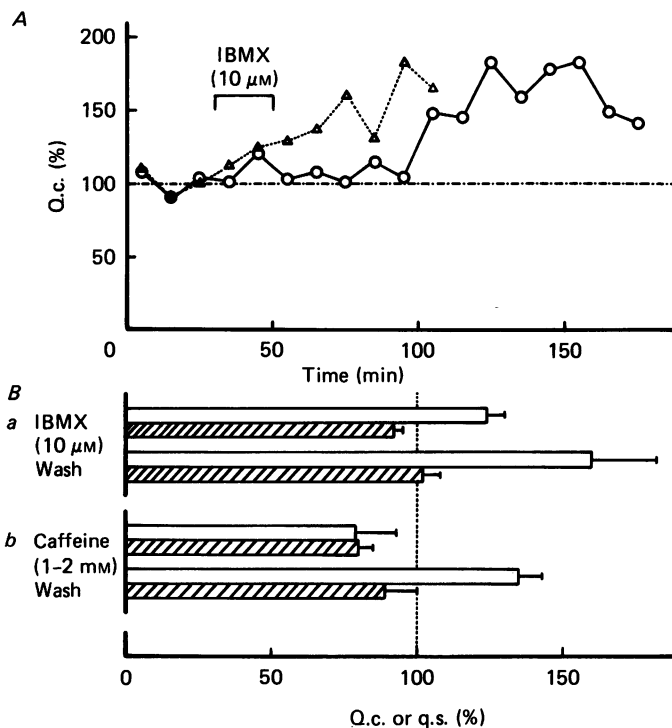

Fig. 8. *A*, the time course of potentiation of quantal content of fast e.p.s.p.s induced by IBMX (10 μM) in two different cells. Each point was expressed as a percentage of the control. IBMX was superfused to the ganglion during the time period marked by a horizontal bar. Note a marked difference in the onset of potentiation between these two cells. *B*, effects of IBMX (10 μM) and caffeine (1–2 mM) on quantal content (q.c.; open column) and size (q.s.; hatched column). Each value, expressed as a percentage of the control, is the mean and s.E. of the mean of data obtained during a 20 min exposure or 30 min after wash in eight cells (both in *a* and *b*).

(Fig. 8Bb). These results can be explained by the idea that blocking the hydrolysis of endogenous cyclic AMP, which is presumably produced at a low rate in the absence of a stimulant in the nerve terminal, causes the accumulation of cyclic AMP in the terminal which results in the activation of the l.t.p. mechanism. The effect of caffeine (but, presumably not the action of IBMX for a low concentration being used) may also be in part accounted for by the Ca^{2+}-mobilizing action (Kuba, 1980), which would have raised free Ca^{2+} in the terminal and subsequently activated the Ca^{2+}-dependent l.t.p. of transmitter release (cf. Koyano *et al.* 1985).

Cholera toxin causes l.t.p. Cholera toxin is known to activate irreversibly adenylate cyclase by the ADP ribosylation of guanosine 5′-triphosphate (GTP)-binding protein (Cassel & Pfeuffer, 1978; Gill & Meren, 1978) for the activation of adenylate cyclase (cf. Lefkowitz, Stadel & Caron, 1983). The exposure of the ganglion to cholera toxin (2 μg ml^{-1}) for 20 min produced the long-lasting enhancement of quantal content (320, 187, 127 and 153 % of the control at 40 min after wash) without a change in quantal size in four cells out of six. In other two cells, only a small potentiation was observed (116 and 120 %). These results, excluding those in two cases, are in accordance with the long-term effects of dibutyryl cyclic AMP, cyclic AMP and phosphodiesterase inhibitors on quantal content, and suggest an involvement of GTP-binding protein in the activation of adenylate cyclase in the preganglionic terminals.

Suppression of adrenaline-induced l.t.p. at low temperature. Adrenaline-induced l.t.p. was markedly suppressed by lowering temperature (to 11–13 °C from room temperature). In contrast, the inhibition of quantal content during treatment was greater at low temperature than at room temperature. This must be due to the suppression of the potentiation which coexisted (but masked by inhibition) during treatment. These results suggest the involvement of a metabolic process in the mechanism of adrenaline-induced l.t.p., which is presumably activated by endogenous cyclic AMP.

Regulation of adrenaline-induced l.t.p.

Dibutyryl cyclic GMP blocks the generation of adrenaline-induced l.t.p. It has been known that in certain cellular processes guanylate cyclase operates antagonistically against an adenylate cyclase system (for instance, see Libet *et al.* 1975; cf. Goldberg & Haddox, 1977). When dibutyryl cyclic GMP (100 μM) was applied simultaneously, adrenaline (10 μM) failed to induced l.t.p. of quantal content in all the cells examined (109 $\pm$ 17 %, $n = 4$, at 30 min after wash: Fig. 9B). However, when dibutyryl cyclic GMP was superfused to the ganglion after the end of application of adrenaline, adrenaline-induced l.t.p. was induced in five cells (244, 147, 125, 123 and 180 % of the control at 30 min: Fig. 9C) out of eight, while the generation of the l.t.p. was blocked in three cells (88, 116 and 82 %). These results indicate that dibutyryl cyclic GMP is less effective in blocking the adrenaline-induced l.t.p. mechanism once cyclic AMP has risen in the presynaptic terminals. It is to be noted that dibutyryl cyclic GMP by itself had no effects on quantal content (Fig. 9A).
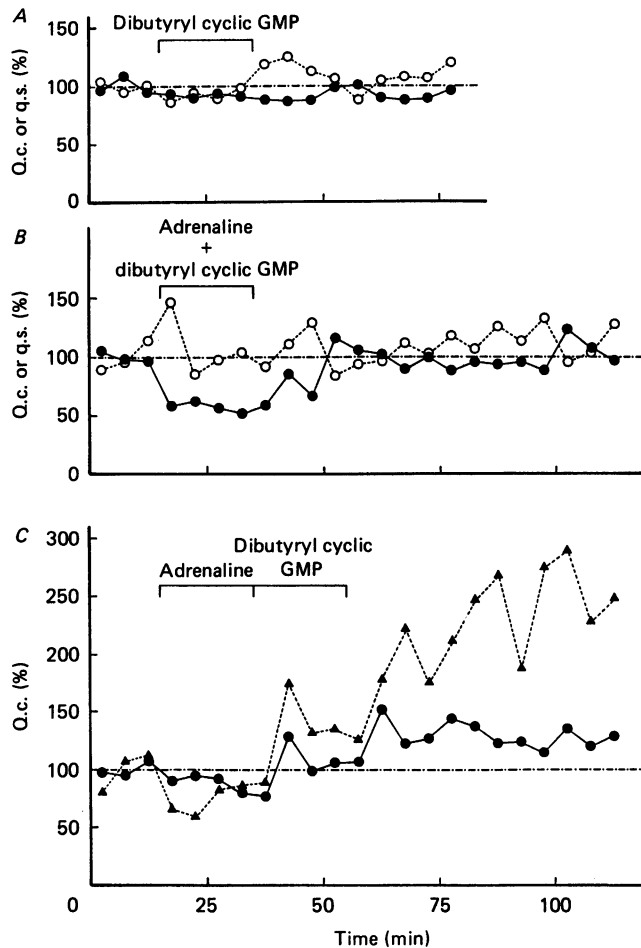
Fig. 9. Effects of dibutyryl cyclic GMP on the adrenaline-induced l.t.p. *A*, effects of dibutyryl cyclic GMP (100 μM) on the quantal content (q.c.) and size (q.s.) of the fast e.p.s.p. during and after treatment. *B* and *C*, effects of dibutyryl cyclic GMP (100 μM) on the l.t.p. induced by adrenaline (10 μM), when the nucleotide was applied concomitantly with adrenaline (*B*), or after exposure to adrenaline (*C*). ● and ▲ indicate quantal content expressed as a percentage of the control, while ○ are quantal size. The drugs were applied during the time period indicated by a horizontal bar. In *C*, two different types of effect on quantal content are shown.

DISCUSSION

Possible mechanisms of adrenaline-induced l.t.p.

The mechanism of adrenaline-induced l.t.p. in bull-frog sympathetic ganglia may be envisaged in the following schema. The binding of adrenaline to a β-adrenoceptor activates adenylate cyclase through the activation of GTP-binding protein. The resultant rise in endogenous cyclic AMP in the presynaptic nerve terminals (cf. Röhrkasten & Zimmermann, 1983) initiates a sequence of metabolic processes which presumably includes the phosphorylation of a specific protein (involved in or modulating a transmitter release mechanism) by cyclic-AMP-dependent protein kinase (Nestler & Greengard, 1982; cf. Cohen, 1982), as suggested for the potentiation

of transmitter release by serotonin in *Aplysia* ganglia (Klein & Kandel, 1980; cf. Kandel & Schwartz, 1982). This results eventually in the long-lasting enhancement in both evoked and spontaneous release of transmitter.

In a cascade mechanism of adrenaline-induced l.t.p., a regulatory process involving endogenous cyclic GMP may operate. It seems likely that endogenous cyclic GMP (which was found to exist in the cholinergic terminals: cf. Röhrkasten & Zimmermann, 1983) inhibits the generation of adrenaline-induced l.t.p., presumably by acting on an initial step of its mechanism. Koketsu & Yamada (1982) reported the existence of a muscarinic receptor inhibiting transmitter release at the preganglionic terminals in bull-frog sympathetic ganglia. Accordingly, it might be possible that the activation of such a muscarinic receptor would raise cyclic GMP level in the terminals (Michaelson, Avissar, Kloog & Sokolovsky, 1979; cf. Goldberg & Haddox, 1977). A similar antagonistic role of cyclic GMP was reported for the mechanism of the long-lasting potentiation of slow e.p.s.p. by dopamine in rabbit sympathetic ganglia (Libet *et al.* 1975).

Bernier, Castellucci, Kandel & Schwartz (1982) found that the time course of the short-term potentiation in *Aplysia* ganglia parallels closely that of an increased level of cyclic AMP. However, this may not be the case for the adrenaline-induced l.t.p. in the sympathetic ganglion. Phosphodiesterase in the preganglionic terminals appears to be active even under the resting condition, as evidenced by the generation of l.t.p. by its inhibitors, IBMX and caffeine. It is unlikely therefore that cyclic AMP or dibutyryl cyclic AMP applied externally for a short period would remain unhydrolysed in the preganglionic terminals over a long period, during which the l.t.p. of transmitter release persisted. Consequently, there may be a process which continues to be activated after a brief rise of endogenous cyclic AMP in the presynaptic terminals of the bull-frog sympathetic ganglion. The final step of the adrenaline-induced l.t.p. mechanism, leading to increased transmitter release, will be reported elsewhere.

Origin of adrenaline for adrenaline-induced l.t.p. in situ

Suetake, Kojima, Inanaga & Koketsu (1981) suggested that depolarization of the post-ganglionic neurone releases catecholamines from the ganglion cell soma. Enhancement in the activity of the pre- and accordingly post-ganglionic neurones may thus raise the catecholamine concentration around the presynaptic terminals and activate the adrenaline-induced l.t.p. mechanism. This hypothesis, however, is not supported by the recent findings that the tetanic stimulation of the pre- (33 Hz, 10 s: Koyano *et al.* 1985) or post-ganglionic (20 Hz, 5 s: Kumamoto & Kuba, 1983*b*) nerve produced a l.t.p. different from adrenaline-induced l.t.p. Nevertheless, this idea remains to be studied, since is is possible that the durations of tetanic stimulation used in the above experiments would not have been long enough to raise and/or keep the catecholamine concentration around the presynaptic terminals, as observed for inability of a 5 min exposure to exogenous adrenaline to produce adrenaline-induced l.t.p. (K. Kuba & E. Kumamoto, unpublished observation).

Another origin of adrenaline would be outside the ganglion. The concentration of adrenaline least effective for inducing adrenaline-induced l.t.p. was as low as $0 \cdot 1 \ \mu M$ (Koketsu *et al.* 1982*a*). It is not known, however, whether the catecholamine concentration in circulating blood rises to this level under physiological conditions.

Physiological significance

The adrenaline-induced l.t.p. in bull-frog sympathetic ganglia would be the first evidence that shows the involvement of a monoamine-cyclic-AMP system in the presynaptic mechanism of synaptic plasticity at vertebrate synapses. Bliss *et al.* (1983) have recently suggested a role of monoamines in the mechanism of l.t.p. in the hippocampus of the rat. Thus, the involvement of catecholamines in a l.t.p. mechanism is not specific to the bull-frog sympathetic ganglion. In this context, the adrenaline-induced l.t.p. could be a general model for the cellular mechanism of learning and memory.

In addition to adrenaline-induced l.t.p., two other types of l.t.p. have been found in bull-frog sympathetic ganglia; one is the l.t.p. of transmitter release induced by Ca^{2+}-dependent activities of the presynaptic nerve terminals (Koyano *et al.* 1985) and the other is the l.t.p. of the sensitivity of the post-synaptic membrane to transmitter caused by Ca^{2+}-dependent post-synaptic activities (Kumamoto & Kuba, 1983*b*). Although it is not known how these three types of l.t.p. co-operate under physiological conditions, there is no doubt that they play important roles in the regulation of the activity of the peripheral autonomic nervous system.

REFERENCES

ANDERSEN, P., SUNDBERG, S. H., SVEEN, O., SWANN, J. W. & WIGSTRÖM, H. (1980). Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea-pigs. *Journal of Physiology* 302, 463–482.

BARANYI, A. & FEHÉR, O. (1981). Synaptic facilitation requires paired activation of convergent pathways in the neocortex. *Nature* 290, 413–415.

BERNIER, L., CASTELLUCCI, V. F., KANDEL, E. R. & SCHWARTZ, J. H. (1982). Facilitatory transmitter causes a selective and prolonged increase in adenosine 3′:5′-monophosphate in sensory neurons mediating the gill and siphon withdrawal reflex in *Aplysia*. *Journal of Neuroscience* 2, 1682–1691.

BLISS, T. V. P. (1979). Synaptic plasticity in the hippocampus. *Trends in Neurosciences* 2, 42–45.

BLISS, T. V. P., GODDARD, G. V. & RIIVES, M. (1983). Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. *Journal of Physiology* 334, 475–491.

BLISS, T. V. P. & LØMO, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology* 232, 331–356.

BRIGGS, C. A., BROWN, T. H. & McAFEE, D. A. (1985*a*). Neurophysiology and pharmacology of long-term potentiation in the rat sympathetic ganglion. *Journal of Physiology* 359, 503–521.

BRIGGS, C. A., McAFEE, D. A. & McCAMAN, R. E. (1985*b*). Long-term potentiation of synaptic acetylcholine release in the superior cervical ganglion of the rat. *Journal of Physiology* 363, 181–190.

BROWN, T. H. & McAFEE, D. A. (1982). Long-term synaptic potentiation in the superior cervical ganglion. *Science* 215, 1411–1413.

CASSEL, D. & PFEUFFER, T. (1978). Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. *Proceedings of the National Academy of Sciences of the U.S.A.* 75, 2669–2673.

COHEN, P. (1982). The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature* **296**, 613–620.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *Journal of Physiology* **124**, 560–573.

DUNANT, Y. & DOLIVO, M. (1968). Plasticity of synaptic functions in the excised sympathetic ganglion of the rat. *Brain Research* **10**, 271–273.

GILL, D. M. & MEREN, R. (1978). ADP-ribosylation of membrane proteins catalyzed by cholera toxin: Basis of the activation of adenylate cyclase. *Proceedings of the National Academy of Sciences of the U.S.A.* **75**, 3050–3054.

GOLDBERG, N. D. & HADDOX, M. K. (1977). Cyclic GMP metabolism and involvement in biological regulation. *Annual Review of Biochemistry* **46**, 823–896.

KANDEL, E. R. & SCHWARTZ, J. H. (1982). Molecular biology of learning: Modulation of transmitter release. *Science* **218**, 433–443.

KATO, E., KOKETSU, K., KUBA, K. & KUMAMOTO, E. (1985). The mechanism of the inhibitory action of adrenaline on transmitter release in bullfrog sympathetic ganglia: Independence of cyclic AMP and calcium ions. *British Journal of Pharmacology* **84**, 435–443.

KLEIN, M. & KANDEL, E. R. (1980). Mechanism of calcium current modulation underlying presynaptic facilitation and behavioral sensitization of Aplysia. *Proceedings of the National Academy of Sciences of the U.S.A.* **77**, 6912–6916.

KOKETSU, K., AKASU, T., MIYAGAWA, M. & HIRAI, K. (1982*a*). Modulation of nicotinic transmission by biogenic amines in bullfrog sympathetic ganglia. *Journal of the Autonomic Nervous System* **6**, 47–53.

KOKETSU, K., MIYAGAWA, M. & AKASU, T. (1982*b*). Catecholamine modulates nicotinic ACh-receptor sensitivity. *Brain Research* **236**, 487–491.

KOKETSU, K. & YAMADA, M. (1982). Presynaptic muscarinic receptors inhibiting active acetylcholine release in the bullfrog sympathetic ganglion. *British Journal of Pharmacology* **77**, 75–82.

KOYANO, K., KUBA, K., KUMAMOTO, E., MINOTA, S. & NOHMI, M. (1983). Synaptic plasticity in sympathetic ganglia. I. Long-term potentiation induced by presynaptic activities. *Journal of the Physiological Society of Japan* **45**, 535.

KOYANO, K., KUBA, K. & MINOTA, S. (1985). Long-term potentiation of transmitter release induced by repetitive presynaptic activities in bull-frog sympathetic ganglia. *Journal of Physiology* **359**, 219–233.

KUBA, K. (1980). Release of calcium ions linked to the activation of potassium conductance in a caffeine-treated sympathetic neurone. *Journal of Physiology* **298**, 251–269.

KUBA, K., KATO, E., KUMAMOTO, E., KOKETSU, K. & HIRAI, K. (1981). Sustained potentiation of transmitter release by adrenaline and dibutyryl cyclic AMP in sympathetic ganglia. *Nature* **291**, 654–656.

KUBA, K. & KOKETSU, K. (1978). Synaptic events in sympathetic ganglia. *Progress in Neurobiology* **11**, 77–169.

KUMAMOTO, E. & KUBA, K. (1983*a*). Independence of presynaptic bimodal actions of adrenaline in sympathetic ganglia. *Brain Research* **265**, 344–347.

KUMAMOTO, E. & KUBA, K. (1983*b*). Sustained rise in ACh sensitivity of a sympathetic ganglion cell induced by postsynaptic electrical activities. *Nature* **305**, 145–146.

LEFKOWITZ, R. J., STADEL, J. M. & CARON, M. G. (1983). Adenylate cyclase-coupled beta-adrenergic receptors: Structure and mechanisms of activation and desensitization. *Annual Review of Biochemistry* **52**, 159–186.

LIBET, B., KOBAYASHI, H. & TANAKA, T. (1975). Synaptic coupling into the production and storage of a neuronal memory trace. *Nature* **258**, 155–157.

MICHAELSON, D. M., AVISSAR, S., KLOOG, Y. & SOKOLOVSKY, M. (1979). Mechanism of acetylcholine release: Possible involvement of presynaptic muscarinic receptors in regulation of acetylcholine release and protein phosphorylation. *Proceedings of the National Academy of Sciences of the U.S.A.* **76**, 6336–6340.

NESTLER, E. J. & GREENGARD, P. (1982). Nerve impulses increase the phosphorylation state of protein I in rabbit superior cervical ganglion. *Nature* **296**, 452–454.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *Journal of Cellular Comparative Physiology* **55**, 15–30.

Röhrkasten, A. & Zimmermann, H. (1983). Enrichment of cyclic adenosine 3′,5′-monophosphate and cyclic guanosine 3′,5′-monophosphate in cholinergic axon terminals of the *Torpedo* electric organ. *Neuroscience Letters* **42**, 201–206.

Suetake, K., Kojima, H., Inanaga, K. & Koketsu, K. (1981). Catecholamine is released from non-synaptic cell-soma membrane: histochemical evidence in bullfrog sympathetic ganglion cells. *Brain Research* **205**, 436–440.