The mechanism of the inhibitory action of adrenaline on transmitter release in bullfrog sympathetic ganglia: independence of cyclic AMP and calcium ions

E. Kato, K. Koketsu, K. Kuba*[1] & E. Kumamoto*

Department of Physiology, Kurume University, School of Medicine, Kurume 830 and Department of Physiology*, Saga Medical School, Nabeshima, Saga 840-01, Japan

1   The effects of adrenaline and dibutyryl adenosine 3':5' - cyclic monophosphate (db cyclic AMP) on nicotinic transmission in bullfrog sympathetic ganglia were compared by use of an intracellular recording technique.

2   The evoked release of transmitter, acetylcholine (ACh), was decreased in the presence of adrenaline (10–100 μM), while the postsynaptic sensitivity to ACh was unchanged (10 μM adrenaline) or slightly reduced (100 μM).

3   Transmitter release was similarly inhibited by dopamine (10 μM), but not by isoprenaline (10 μM). The inhibitory action of adrenaline on transmitter release was blocked by phenoxybenzamine but not by propranolol.

4   The inhibition of transmitter release was independent of the external calcium concentration.

5   The evoked release of transmitter and the electrical properties of the postsynaptic membrane were unchanged during exposure to db cyclic AMP (1–4 mM), while the postsynaptic sensitivity to ACh was slightly but significantly depressed.

6   The spontaneous release of transmitter in a high K^+ (10 mM) solution was decreased in the presence of adrenaline (100–300 μM), but unchanged with db cyclic AMP (4 mM).

7   In contrast to the effects during exposure, both the evoked and spontaneous release of transmitter were enhanced after the removal of adrenaline or db cyclic AMP.

8   Neither adrenaline (100 μM) nor db cyclic AMP (4 mM) affected the presynaptic spike and synaptic delay.

9   It is concluded that adrenaline mainly inhibits the release of ACh from the presynaptic terminals through its α-action, while db cyclic AMP reduces slightly the postsynaptic sensitivity to ACh and that both agents facilitate transmitter release when they are removed from the presynaptic terminals. It is further suggested that the inhibitory action of adrenaline is independent of endogenous cyclic AMP and calcium ions.

Introduction

Catecholamines are known to modulate the release of transmitter at several synapses. At the neuromuscular junction, transmitter release is facilitated by noradrenaline (Jenkinson, *et al.*, 1968, Kuba, 1970; Kuba & Tomita, 1971). This facilitatory effect was reported to be mediated by endogenous cyclic AMP (adenosine 3':5'-cyclic monophosphate: Miyamoto & Breckenridge, 1974), which is known to act as an intracellular second messenger. On the other hand, adrenaline and dopamine inhibit transmitter release in mammalian

ganglia (Christ & Nishi, 1971a; Dun & Nishi, 1974). Accordingly, a question arises as to whether these inhibitory actions of catecholamines are mediated by endogenous cyclic AMP. Furthermore, evidence has accumulated recently that adrenaline depresses Ca^{2+}-dependent action potentials in several neurones (Minota & Koketsu, 1977; Horn & McAfee, 1980). It is, therefore, possible that the inhibitory effect of adrenaline on the presynaptic terminals might be caused by the reduction of Ca^{2+} influx during a presynaptic spike.

Our preliminary experiments (Kuba *et al.*, 1981)

[1]Correspondence

have shown that adrenaline causes bimodal actions on transmitter release in bullfrog sympathetic ganglia: inhibition during exposure to adrenaline and long-lasting potentiation after its removal. In this paper, we first describe in detail the mode of action of adrenaline in producing its inhibitory effects on the preganglionic nerve terminal in bullfrog sympathetic ganglia. Then, the evidence against the involvement of endogenous cyclic AMP and Ca^{2+} in the inhibitory mechanism is presented. The mechanism of the long-lasting potentiation after the removal of adrenaline will be described separately in detail.

Methods

The ninth or tenth lumbar sympathetic ganglia of bullfrogs (*Rana catesbeiana*) were isolated. Intracellular recordings were made from B-type neurones (Nishi & Koketsu, 1960). Intracellular electrodes were filled with 3 M KCl or 1–3 M K citrate (tip resistance, 25–100 MΩ). A Wheatstone bridge circuit was used to pass current to the membrane through a recording electrode in each experiment except for voltage-clamp experiments.

Composition of the bathing (Ringer) solution was as follows (mM): NaCl 112, KCl 2.0, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4. A low Ca^{2+}-high Mg^{2+} solution was prepared by reducing $CaCl_2$ (0.7–0.9 mM) and adding $MgCl_2$ (5.4–6.5 mM) in normal Ringer (the tonicity was adjusted by alteration of the NaCl concentration). All experiments were carried out at room temperature (20–24°C). Data were examined for statistical significance by Student's *t* test.

Fast excitatory postsynaptic potentials (fast e.p.s.ps) were recorded in a low Ca^{2+}-high Mg^{2+} solution in most experiments and their quantal content (q.c.) and size were calculated from 100–200 fast e.p.s.ps, using the Variance or Failure Method (del Castillo & Katz, 1954; see also, Kato & Kuba, 1980). The quantal content calculated by these two different methods agreed well in most experiments (correlation coefficient between these values in pooled data was greater than 0.99), when they were less than 3.0. In some experiments, the fast e.p.s.ps were recorded in normal Ringer or high Ca^{2+} solution, both of which contained (+)-tubocurarine (30 μM). Miniature e.p.s.ps (m.e.p.s.ps) were recorded in a high K^+ (10 mM) solution to raise the basal frequency. The acetylcholine (ACh) sensitivity of the cell membrane was measured by recording nicotinic ACh potentials produced by iontophoretic application of ACh (2 M) through a microelectrode (tip resistance, 20–100 MΩ) in the presence of atropine (28 μM).

The voltage-clamp device used for recording the synaptic current (fast e.p.s.c.) underlying the fast e.p.s.p. was similar to that described by Kuba & Nishi (1979). Two microelectrodes were inserted into a single ganglion cell; one filled with 3 M KCl (25–50 MΩ) for recording membrane potential and the other filled with 1 M K citrate (25–50 MΩ) for passing current. The presynaptic nerve action current, together with postsynaptic responses, was recorded by an extracellular electrode filled with 1 M Na citrate as described previously (Ginsborg, 1971; Kato & Kuba, 1980).

Adrenaline, dopamine and propranolol were obtained from Sigma Co., db cyclic AMP (sodium salt) from P-L Biochemical Co., (+)-tubocurarine, isoprenaline and phenoxybenzamine from Nakarai Chemical Co., Japan.

Results

Presynaptic inhibition induced by adrenaline

Superfusion with adrenaline (100 μM) decreased markedly the amplitude of the fast e.p.s.p. recorded in a low Ca^{2+}-high Mg^{2+} solution in a few minutes (Figure 1A). Likewise, the quantal content of the fast e.p.s.p. (q.c.: 0.30–2.43 under control conditions) was reduced to $52.0 \pm 7.0\%$ (mean ± s.e.mean, $n = 14$, $P < 0.001$, after perfusion for 5 min) of the control with a small decrease in the quantal size ($88.0 \pm 5.6\%$, $n = 14$, $0.05 < P < 0.1$). The inhibition of quantal content was smaller ($68.0 \pm 4.0\%$, $n = 24$, $P < 0.001$; control q.c., 0.47–4.12) at a lower concentration (at 10 μM) with little change in quantal size ($97.0 \pm 4\%$, $n = 24$, $P > 0.4$). After the removal of adrenaline, the amplitude and quantal content of the fast e.p.s.p. recovered to the control values and subsequently rose to a greater value in most cells (Figure 1A: see Kuba *et al.*, 1981). Similar effects were observed for the amplitudes of the fast e.p.s.ps recorded in Ringer solution containing (+)-tubocurarine.

Figure 1B shows the effect of adrenaline (100 μM) on the synaptic current (fast e.p.s.c.) underlying the fast e.p.s.p. In this cell, adrenaline decreased the amplitude of fast e.p.s.c. to 52% of the control without a change in time course. Similar suppressions were observed in other two cells (68 and 71%). A potentiation after the removal of adrenaline, as was observed in the fast e.p.s.p. was not detected, perhaps because the period of observation after cessation of exposure to isoprenaline was insufficiently long.

ACh potentials tended to be depressed in amplitude during treatment with adrenaline at a low concentration (10 μM: $91.3 \pm 3.7\%$, $n = 8$, $0.1 > P > 0.05$: Figure 1D), but significantly decreased at a high concentration (100 μM: $81.9 \pm 6.3\%$, $n = 9$, $0.05 > P > 0.02$: Figure 1C: cf. Koketsu *et al.*, 1982b), although a difference between the reductions at two concentrations was not significant ($P > 0.2$). Resting
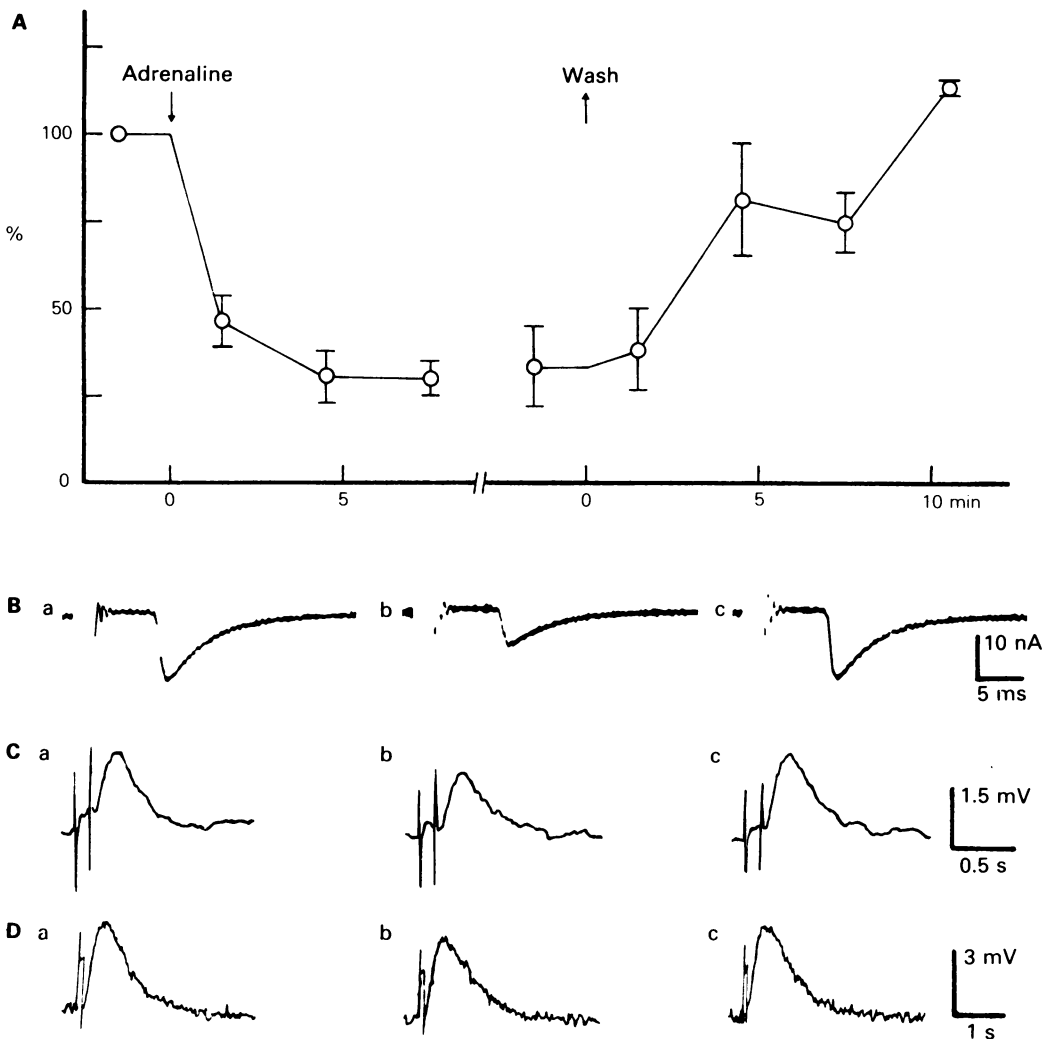
Figure 1 Effects of adrenaline on the fast e.p.s.ps, fast e.p.s.cs and ACh potentials. (A) The time course of changes in the amplitude of fast e.p.s.ps before, during and after the application of adrenaline (100 μM). The mean amplitude of sixty fast e.p.s.ps recorded every 3 s in a low Ca^{2+}-high Mg^{2+} solution were normalized to the control values. The average of normalized values (± s.e.mean) obtained from six cells was plotted against time after the beginning or end of exposure to adrenaline. Since the duration of exposure to adrenaline was varied in some experiments (10–20 min), the data during exposure were plotted only for those up to 10 min. Adrenaline 100 μM was applied at the downward arrow and removed at the upward arrow. (B) Fast e.p.s.cs recorded by the two-electrodes voltage clamp technique before (a), during (b) and 5 min after (c) exposure to adrenaline (100 μM) for 5 min. (C & D) ACh potentials induced by iontophoretic application of ACh before (a), during (b) and after (c) treatment with adrenaline (100 μM in (C); 10 μM in (D)).

membrane potential and resistance of the ganglion cell were not significantly affected by adrenaline (10–100 μM). These results indicate that adrenaline inhibits the evoked release of transmitters during its presence around the presynaptic terminals, while the postsynaptic effect is notable only at a high concentration (cf. Koketsu et al., 1982b).

The type of adrenoceptor involved in the presynaptic inhibition

Dopamine (10 μM) had effects similar to those of adrenaline (10 μM), as it reduced the quantal content of the fast e.p.s.p. to 66.8 ± 10.0%, ($n = 8$, $P < 0.02$: control q.cs, 0.83–4.92) during exposure and
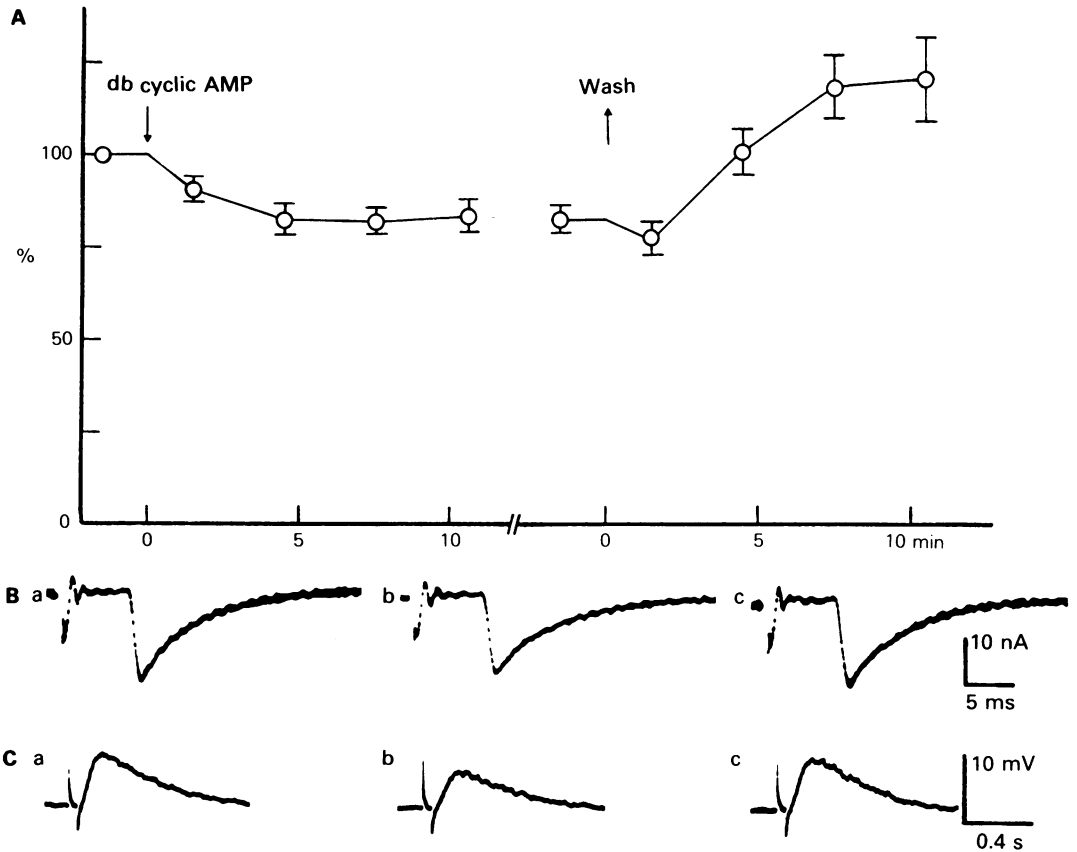
Figure 2 Effects of dibutyryl cyclic AMP (db cyclic AMP) on the fast e.p.s.ps, fast e.p.s.cs and ACh potentials. (A) Time course of changes in the amplitude of fast e.p.s.p. before, during and after exposure to db cyclic AMP (4 mM). Data were obtained in low Ca^{2+}-high Mg^{2+} solutions from 22 cells and plotted as shown in Figure 1A. (B) Fast e.p.s.cs before (a), during (b) and 5 min after (c) treatment with db cyclic AMP (4 mM) for 5 min. (C) ACh potentials before (a), during (b) and 5 min after (c) exposure to db cyclic AMP (4 mM) for 5 min.

sometimes an enhancement of the fast e.p.s.ps was seen after it was washed out. On the other hand, isoprenaline (10 μM) produced an enhancement of the quantal content (185.0 ± 25.0%, $n = 4$, $P < 0.05$: control q.cs, 0.57–1.80) even in the presence of the drug. Furthermore, the depressant effect of adrenaline (10 μM) was not seen (107.2 ± 9.3%, $n = 6$; control q.cs, 0.98–2.82) in the presence of phenoxybenzamine (10 μM), but observed (70.0 ± 10.6%, $n = 4$, $0.05 < P < 0.1$: control q.cs, 0.76–4.5) in the presence of propranolol (10 μM). Thus, the α-adrenoceptor is involved in the inhibitory action of adrenaline on the presynaptic terminals, as has been suggested for mammalian ganglia (Christ & Nishi, 1971).

Lack of involvement of cyclic AMP in the inhibition

In contrast to the action of adrenaline, db cyclic AMP

(1–4 mM) reduced only slightly the amplitude of the fast e.p.s.p. (Figure 2A). This suppression was not due to a presynaptic effect of the cyclic nucleotide, but to a postsynaptic effect, because under these conditions the quantal content was unaffected (103.0 ± 6.8%, $n = 18$: control q.cs, 0.57–2.95), while the quantal size was significantly reduced (84.7 ± 4.4%, $n = 18$, $P < 0.01$). When the ganglion was washed with a nucleotide-free solution, the amplitude of the fast e.p.s.p. recovered to the control value and subsequently rose to a considerable magnitude (Figure 2A). The after-potentiation was accompanied by an increased quantal content (cf. Kuba *et al.*, 1981).

The amplitude of the fast e.p.s.c. was slightly depressed without a change in time course during exposure to db cyclic AMP (4 mM) in all the cells studied (84.5 ± 6.5%, $n = 6$, $0.1 > P > 0.05$: Figure 2B). The after-potentiation was not seen as there was

Table 1   Effects of adrenaline and dibutyryl cyclic AMP (db cyclic AMP) on m.e.p.s.ps recorded in a high K$^+$ (10 mM) solution

	Frequency mean ± s.e.	n	P	Amplitude mean ± s.e.	P
Adrenaline 100 μM	68.6 ± 9.1%	7	<0.02	83.3 ± −7.9%	<0.1
Adrenaline 10 μM					
(group A)	132.0 ± 14.0	4	<0.05	109.0 ± 12.0	>0.4
(group B)	72.1 ± 7.8	8	<0.01	96.9 ± 6.9	>0.5
db cyclic AMP	95.0 ± 5.0	14	>0.3	98.6 ± 3.8	>0.5
(1–4 mM)					

M.e.p.s.ps were sampled for analysis for a period of 5–10 min before or 5 min after application of a drug. Group A or B indicates a group of cells which showed an increase or decrease, respectively, in the presence of adrenaline.

only a short period of observation after the removal of the nucleotide.

Db cyclic AMP (4 mM) decreased reversibly the amplitude of ACh potentials (80.3 ± 3.3%, $n = 10$, $P < 0.001$: Figure 2C) without changes in the resting potential and input resistance of the ganglion cell. This effect was very fast (appearing in a few minutes) and swiftly reversible. These results provide support for the idea that the nucleotide acts postsynaptically and further suggest that the inhibitory action of adrenaline on the evoked release is not mediated by endogenous cyclic AMP.
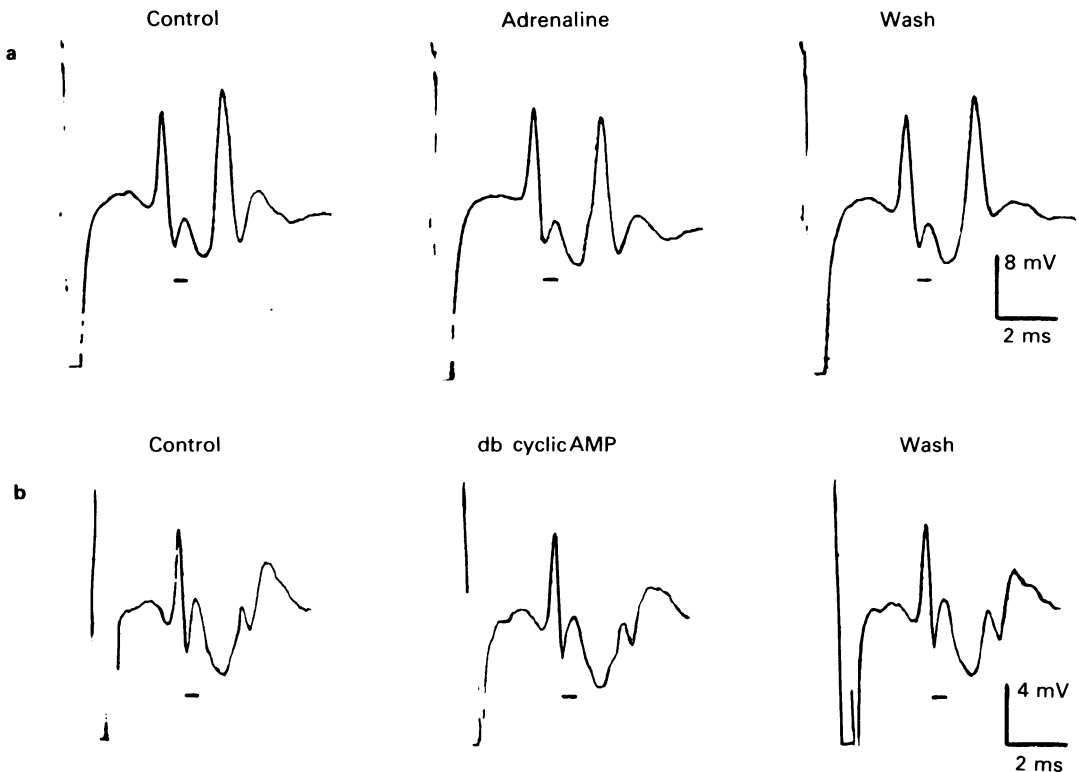

Figure 3   Effects of adrenaline (100 μM) (a) and dibutyryl cyclic AMP (db cyclic AMP 4 mM) (b) on the presynaptic terminal spike and postsynaptic currents recorded with an extracellular microelectrode. Horizontal bars indicate the synaptic delay. All the records are the electronic summations of twenty records. The records during and after exposure to the drugs were taken 5 min after exchange of a perfusing solution.

Mode of the presynaptic inhibition

When adrenaline (100–300 μM) was applied to the ganglion for a short period (10 min), the frequency of miniature e.p.s.ps (m.e.p.s.ps) in a raised K^+ concentration was decreased significantly with a small decrease in amplitude (Table 1, first line). Adrenaline, applied at relatively low concentrations (2.5–50 μM) for a long time (20–30 min), increased m.e.p.s.p. frequency during exposure in four cells out of twelve (Table 1, second line), whereas it decreased the frequency in the remaining cells (third line). In many cells, the m.e.p.s.p. frequency was potentiated after the removal of adrenaline (Kuba et al., 1981). By contrast, db cyclic AMP (1–4 mM) did not affect the m.e.p.s.p. frequency and decreased slightly the amplitude during application (Table 1, fourth line). However, the frequency of m.e.p.s.ps increased significantly after the removal of db cyclic AMP, as seen in the action of adrenaline.

Adrenaline (100 μM) affected neither the amplitude of the action current (104.5 ± 4.3%, n = 11) of the presynaptic terminal nor the synaptic delay (102.0 ± 2.6%, n = 11), which was measured from the negative peak of a presynaptic spike to the beginning of the postsynaptic current (Figure 3A). These parameters remained unchanged at least 5 min after the removal of adrenaline (102.7 ± 7.2%, n = 6;

100.6 ± 4.7%, n = 6, respectively). Similarly, db cyclic AMP (4 mM) did not alter the presynaptic spike and synaptic delay during (97.6 ± 2.9%, n = 7; 103.4 ± 1.9%, n = 7, respectively) and 5 min after its application (96.7 ± 4.6%, n = 6; 97.1 ± 2.6, respectively), as seen in Figure 3B.

The inhibitory actions of adrenaline on the fast e.p.s.p. at normal (1.8 mM) or high Ca^{2+} (7.2 mM) concentration did not differ from that observed in a low Ca^{2+}-high Mg^{2+} solution (Figure 4). The extent of reductions in amplitude of the fast e.p.s.ps was essentially similar in three different concentrations of Ca^{2+} for the effects of two different concentrations of adrenaline.

Discussion

The results of the present experiments demonstrated that adrenaline inhibits both the evoked and spontaneous release of transmitter in bullfrog sympathetic ganglia through the activation of α-adrenoceptors. Furthermore, it became evident that this action is not mediated by endogenous cyclic AMP.

There are several possible mechanisms for the presynaptic inhibitory action of adrenaline. First, adrenaline may cause a change in the resting membrane potential of the presynaptic terminal, which results in the depression of transmitter release. However, this seems unlikely. Hyperpolarization of the presynaptic terminal membrane should decrease the frequency of m.e.p.s.p. especially in a high K^+ solution (a reduction in frequency was, indeed, observed in the presence of adrenaline) but not the quantal content of the fast e.p.s.p. (cf. Takeuchi & Takeuchi, 1962). On the other hand, if depolarization took place at the presynaptic terminals in the presence of adrenaline, the m.e.p.s.p. frequency should have increased; this was not, however, consistently observed during exposure. Moreover, the absence of a change in the presynaptic membrane potential can be indicated by the unaltered amplitude of a presynaptic spike in the presence of adrenaline.

Secondly, adrenaline might lower the basal level of the intracellular free Ca^{2+} in the presynaptic terminals, thereby reducing both the evoked and spontaneous transmitter release. Such a mechanism may be achieved by the enhancement of the intracellular sequestration of Ca^{2+} or active extrusion of Ca^{2+}. Evidence for enhancement by adrenaline of a Ca^{2+} uptake mechanism has indeed been obtained for the sarcoplasmic reticulum (cf. Tada et al., 1978). Such an effect would be highly temperature-dependent. However, the inhibitory action of adrenaline was not depressed by lowering the temperature (Kumamoto & Kuba, 1983). The action of adrenaline which promotes Ca^{2+} uptake into the sarcoplasmic reticulum of a slow
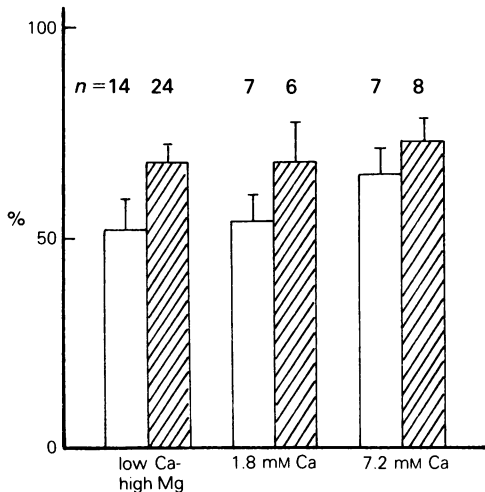

Figure 4 The magnitude of the inhibitory action of adrenaline on the fast e.p.s.p. at different concentrations of external Ca^{2+}. Ordinate scale indicates the quantal content (low Ca^{2+}-high Mg^{2+}; 0.7–0.9 mM and 5.4–6.5 mM, respectively) or amplitude (normal or high Ca^{2+}; 1.8 or 7.2 mM, respectively) of the fast e.p.s.p. in the presence of adrenaline (100 μM for open columns; 10 μM for hatched columns) expressed as a relative value to the control.

fibre in the rabbit skeletal muscle is mediated by endogenous cyclic AMP (cf. Tada, et al., 1978), which is inconsistent with the inhibitory action in bullfrog sympathetic ganglia. Furthermore, if this mechanism were responsible, the inhibitory action of adrenaline would occur with a slower onset and decay. The action of adrenaline appeared with a minimal delay, as if it acts as soon as it reaches the nerve terminals, and disappears immediately after its removal.

Thirdly, adrenaline may reduce the increase in Ca^{2+} conductance (G_{Ca}) of the presynaptic membrane which occurs during the generation of a spike. This may be a reasonable assumption, since such an action of adrenaline was first observed in the postsynaptic neurones of bullfrog sympathetic ganglion (Minota & Koketsu, 1977) and later confirmed in mammalian ganglia (Horn & McAfee, 1980). The reduction of m.e.p.s.p. frequency by adrenaline may be consistent with this hypothesis, since m.e.p.s.ps were recorded at an increased K^+ concentration which would have raised the G_{Ca}, although it can also be explained by other mechanisms. If adrenaline inhibits an increase in the G_{Ca} of the presynaptic membrane, the magnitude of the inhibition of transmitter release would depend on the extracellular Ca^{2+} concentration. The computation in the Appendix predicts that if the increase in intraterminal Ca^{2+} concentration produced by the maximum Ca^{2+} influx were assumed to be large relative to the overall dissociation constant for the intracellular Ca^{2+}-buffering activity, the inhibition of the quantal release by adrenaline would become smaller with an elevation in external Ca^{2+} (this was indeed reported for the presynaptic action of adrenaline in mammalian sympathetic ganglia; Christ & Nishi, 1971b). On the other hand, it was a biphasic function of the extracellular Ca^{2+} concentration in computation, if the maximum Ca^{2+} influx was assumed to be relatively small. In contrast to these predictions, the extent of the inhibition observed in the experiments was almost independent of the external Ca^{2+} concentration. This result may cast some doubt on the idea that the blocking effect of adrenaline on the Ca^{2+} channel found at the postsynaptic neurone occurs at the presynaptic terminal and causes presynaptic inhibition.

One of the remaining possibilities for the mechanism of the inhibitory action of adrenaline would be the depressant action on a step in a sequence of excitation-secretion coupling. However, we have no direct evidence for this mechanism which must be the object of future studies.

The effects of adenaline and db cyclic AMP after their removal, together with the findings in previous studies (cf. Kuba et al., 1981; Kumamoto & Kuba, 1983) suggest that in addition to an inhibitory action, adrenaline possesses a long-lasting facilitatory action on transmitter release, which appears to be mediated by endogenous cyclic AMP and a metabolic process related to it. The least concentration of adrenaline in producing the facilitatory effect is as low as 0.1 μM (cf. Koketsu et al., 1982a), while that for the inhibitory action is greater than several μM. Thus, under physiological conditions, the facilitatory effect seems to be predominant and serves to maintain a high level of activity of the peripheral sympathetic nervous system. On the other hand, the physiological role of the inhibitory mechanism would be to prevent the level of sympathetic activity from increasing to a pathological range.

Appendix

Calcium-dependence of the presynaptic inhibition induced by the blockade of the calcium channel

If the opening of the Ca^{2+} channel at the presynaptic terminals is blocked by adrenaline as observed in postganglionic neurones (Minota & Koketsu, 1977; Horn & McAfee, 1980), the release of transmitter induced by a nerve impulse would decrease. How the magnitude of this type of the presynaptic inhibition is affected by changes in the extracellular Ca^{2+} concentration (Ca_o) is examined here.

Invasion of a presynaptic nerve terminal by an action potential opens Ca^{2+} channels, which results in Ca^{2+} influx into the terminal. Assuming that the Ca^{2+} influex is proportional to Ca_o and to the fraction of Ca^{2+} channels bound with Ca^{2+} (Hagiwara & Takahashi, 1967; cf. Hagiwara & Byerly, 1981), the concentration of Ca^{2+} loaded to the terminals (Ca_L) by a single action potential may be expressed by

$$Ca_L = Ca_{L(max)} Ca_o/(Ca_o + Kc) \qquad (1)$$

where $Ca_{L(max)}$ is the maximum Ca^{2+} concentration which can be loaded, Kc is the dissociation constant for the Ca-binding site of the Ca^{2+} channel.

Ca^{2+} thus loaded would be buffered by an intracellular Ca^{2+}-buffering system by the reaction, $Ca^{2+} + B \rightleftharpoons CaB$, where B is the free buffer and CaB is a Ca-buffer complex (cf. Nachshen & Drapeau, 1982). It is assumed, for simplicity, that only a single type of Ca^{2+}-buffering system is operative and that it is in an equilibrium state throughout the process of excitation-secretion coupling (although this may not be so during such a fast process, the results of computation would essentially be the same without this assumption). Thus, as described by Nachshen & Drapeau (1982), free intracellular Ca^{2+} concentration effective for transmitter release (Ca_i) would be

$$Ca_i = (Ca_L - Bt - Kb + ((Bt + Kb - Ca_L)^2 + 4Ca_L Kb)^{1/2})/2 \qquad (2)$$

where Kb is the dissociation constant for CaB and Bt is the total concentration of buffer.

Release of transmitter would occur by the binding of intracellular Ca_i to a macromolecule (X) essential for exocytosis. Accordingly, the release of transmitter (T), expressed by a fraction of the maximum release, would be

$$T = 1/(1 + Kx/Ca_i) \qquad (3)$$

where Kx is the dissociation constant for CaX. Without assuming a cooperative action of Ca_i, the cooperative relationship between Ca_o and transmitter release is obtained (cf. Nachshen & Drapeau, 1982).

Using the equations (1), (2) and (3), the effects of changes in Ca_o on the magnitude of the presynaptic inhibition caused by the blockade of Ca^{2+} channel were simulated. In computation, the value for Kb was assumed to be $0.1-0.5\,\mu M$ which was taken from the value for the active Ca^{2+} transport at the endoplasmic reticulum in the brain synaptosome (Blaustein *et al.*, 1978). The ranges for Kc, Kx and $Ca_{L(max)}$ are restricted by the condition that the computed amount of transmitter release should increase more than ten times with an increase in Ca_o from 0.4 mM to 7.2 mM, as seen in the experiments (cf. Kuba & Koketsu, 1978). Thus, the value for Kc must be close to a value in the midst of a range of variation in Ca_o and was, therefore, assumed to be 2 mM. Kx/Kb must be large enough to allow reconstruction of the steep relationship between transmitter release and Ca_o. However, Kx would be less than $100\,\mu M$, considering other intracellular Ca^{2+}-dependent processes, e.g., muscle contraction (a half maximum tension of the cardiac muscles occurs at the Ca_i of $2-6\,\mu M$; cf. Fabiato, 1982). Then, Kx was assumed to be $2-100\,\mu M$. Using a wide range of values for $Ca_{L(max)}$ $(5-500\,\mu M)$, it was found that there is an optimum value for this constant to obtain the largest slope of the relationship between transmitter release and Ca_o (cf. Nachshen & Drapeau, 1982). Consequently, $Ca_{L(max)}$ values that provided a more than ten times change in release with an increase in Ca_o from 0.4 mM to 7.2 mM were used for computation. The value of Bt was arbitrarily assumed to be $50\,\mu M$.

If adrenaline reduces the number or conductance of opened Ca^{2+} channels, this effect can be simulated by decreasing $Ca_{L(max)}$ in computation. Figure 5 shows changes in the magnitude of the inhibition of transmitter release at various Ca_o. In each computation with a different initial value for $Ca_{L(max)}$, the action of adrenaline was mimicked by
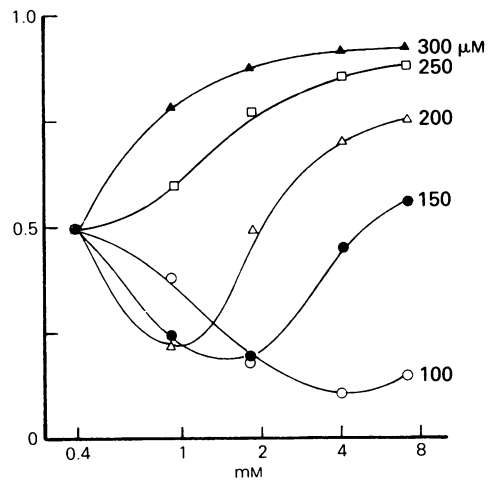

Figure 5 Simulated relationships between the magnitude of the adrenaline inhibition of evoked release and the external Ca^{2+}, assuming that adrenaline reduces Ca^{2+} influx during an action potential. Ordinate scale is the amount of evoked release during a partial blockade of Ca^{2+} influx, which was expressed by a relative value to the control. Abscissa scale is the external Ca^{2+} concentration. Values shown in the right hand side of the curves are the maximum load of Ca^{2+} into the terminal during a nerve impulse. $Kc = 2\,mM$, $Bt = 50\,\mu M$, $Kb = 0.5\,\mu M$, $Kx = 100\,\mu M$.

reducing the $Ca_{L(max)}$ so as to cause 50% inhibition of release at 0.4 mM Ca_o. When $Ca_{L(max)}$ is greater than $238\,\mu M$, the inhibition of transmitter release increases with a rise in Ca_o. In a range between 100 and $238\,\mu M$, the magnitude of the inhibition is biphasic. When $Ca_{L(max)}$ is smaller than $100\,\mu M$, the magnitude of inhibition decreases monotonically with a reduction in Ca_o. These relationships are essentially similar, even when the values for various constants are varied in certain ranges (see above). Thus, if adrenaline reduces the Ca^{2+} influx during an action potential at the presynaptic terminal, the magnitude of the inhibition of transmitter release should vary with Ca_o.

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