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EFFECTS OF DIBUTYRYL CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE AND THEOPHYLLINE ON THE BULLFROG SYMPATHETIC GANGLION CELLS

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1 Effects of dibutyryl cyclic adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) and theophylline on bullfrog sympathetic ganglion cells were examined in order to test the hypothesis that cyclic AMP is essential for the generation of slow inhibitory postsynaptic potentials (i.p.s.ps) in these cells.

2 In the absence or presence of theophylline, dibutyryl cyclic AMP did not hyperpolarize but rather tended to depolarize ganglia that were hyperpolarized by adrenaline.

3 Theophylline augmented neither the P-potential (slow i.p.s.p.) nor adrenaline-induced hyperpolarization.

4 Thus, cyclic AMP does not seem to be essential for the generation of the slow i.p.s.p., at least in amphibian sympathetic ganglion cells.

Introduction

Slow potential changes associated with synaptic activation of sympathetic ganglia have been reported by many authors and attempts have been made to confirm the suggestion of Eccles & Libet (1961) that a catecholamine may be the mediator of the hyperpolarizing potential. The latter potential is recorded extracellularly as the P-potential and intracellularly as the slow inhibitory postsynaptic potential or slow i.p.s.p. Membrane hyperpolarizations induced by adrenaline and dopamine have been observed in mammalian (Libet & Kobayashi, 1969; Kobayashi & Libet, 1970; Libet & Tosaka, 1970; 1971; Christ & Nishi, 1971; Dun & Nishi, 1974) and amphibian (Nakamura & Koketsu, 1972; Weight, 1973; Libet & Kobayashi, 1974; Koketsu & Nakamura, 1976) sympathetic ganglia. However, the mechanisms involved in the production of the slow i.p.s.p. or these catecholamine-induced hyperpolarizations are not entirely clear.

The main aims of this investigation were to test the hypothesis of McAfee & Greengard (1972) that adenosine 3′,5′-monophosphate (cyclic AMP) plays an essential role in the generation of the slow i.p.s.p. and catecholamine-induced hyperpolarizations of sympathetic ganglia and to compare the ionic mechanisms underlying cyclic AMP-induced changes in membrane potential of ganglion cells with those responsible for the catecholamine-induced responses. On the basis of the work of McAfee & Greengard (1972) dibutyryl cyclic AMP would be expected to induce a hyperpolarization. Kuba & Nishi (1976), on the other hand, reported that dibutyryl cyclic AMP did not hyperpolarize bullfrog sympathetic ganglion cells. Since the latter authors had observed hyperpolarizing responses to caffeine, it was of interest to examine the effect of another methylxanthine, theophylline, which is known to inhibit phosphodiesterase activity and which, therefore, might be expected to enhance the effects of endogenously produced cyclic AMP and its administered dibutyryl derivative.

The present experiments were therefore carried out in order to examine the effect of dibutyryl cyclic AMP and theophylline on bullfrog sympathetic ganglion cells, for the purpose of clarifying whether cyclic AMP is essential for the generation of the slow i.p.s.p. or adrenaline-induced hyperpolarization.

Method

Paravertebral sympathetic ganglion chains isolated from bullfrogs (*Rana catesbeiana*) were used in the present experiments. The membrane potential changes of sympathetic ganglion cells were recorded from ganglion-postganglionic nerve preparations by use of sucrose-gap methods (Koketsu & Nishi, 1968; Nishi & Koketsu, 1968b; see also Kosterlitz, Lees & Wallis, 1968). The membrane potential changes of ganglion cells were also recorded by intracellular microelectrodes inserted into the ganglion cell-bodies (Nishi & Koketsu, 1960). Microelectrodes were filled with

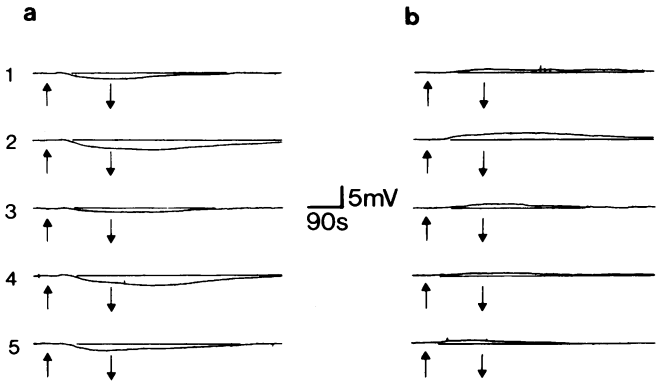

Figure 1 Effects of (a) adrenaline (adrenaline-induced hyperpolarizations) and (b) dibutyryl cyclic AMP on sympathetic ganglia; all records were obtained by the sucrose-gap method, and moments of application and withdrawal of these drugs are indicated by upward and downward arrows, respectively. (a and b) Records 1, 2 and 3 were obtained from the same preparation, and records 4 and 5 were from nicotine-treated and curarized ganglia, respectively. Records 3 were obtained in the presence of theophylline (2 mM). (a) Adrenaline, 1 mM for record 2, 0.3 mM for other records; (b) dibutyryl cyclic AMP, 3 mM for record 2, 1 mM for other records.

3 M KCl and those with resistances in the range 20–30 megaohms were selected for the present experiment.

Ionic compositions of solutions used in the present experiment were as follows: the Ringer solution (mM): NaCl 112, KCl 2, $CaCl_2$ 1.8 and $NaHCO_3$ 2; the K^+-free solution (mM): NaCl 112, $CaCl_2$ 1.8 and $NaHCO_3$ 2. Preparations were continuously superfused with one of the solutions flowing through a preparation-trough (50 × 5 × 4 mm) at the rate of 0.2 ml/second.

Drugs used were as follows: dibutyryl cyclic AMP, sodium (P-L Biochemicals, Inc.), theophylline (Wako Pure Chemical Ind., Japan), (−)-adrenaline bitartrate (SIGMA), nicotine sulphate (Katayama Chemical Ind., Japan), and (+)-tubocurarine chloride ((+)-Tc) (Wako Pure Chemical Ind. Japan).

All experiments were carried out at room temperature (20–22°C).

Results

Effects of dibutyryl cyclic AMP on the membrane potential

The effect of dibutyryl cyclic AMP on the membrane potential of ganglion cells was tested by use of the sucrose-gap method, in those ganglia in which it was confirmed that adrenaline (0.3 mM) induced a hyperpolarization (cf. Nakamura & Koketsu, 1972; Koketsu & Nakamura, 1976). No hyperpolarization was observed when dibutyryl cyclic AMP (1 mM) was applied to these ganglia (10 preparations); rather, the ganglia tended to depolarize (less than 1 mV) and notable depolarizations (about 2 mV) occurred when bibutyryl cyclic AMP was used in a concentration of 3 mM, which was the highest concentration used in these experiments. Effects of dibutyryl cyclic AMP on the membrane potential were also examined in the presence of nicotine (0.24 mM) or (+)-Tc (0.14 mM) because the slow i.p.s.p. can be recorded in the presence of nicotine or (+)-Tc. Dibutyryl cyclic AMP (1 mM) never hyperpolarized the ganglion cells in these nicotinized or curarized ganglia. Examples of these experiments are shown in Figure 1.

The effect of dibutyryl cyclic AMP on the membrane potential of ganglion cells was also examined in the presence of theophylline which is known to be a phosphodiesterase inhibitor. Ganglion cells were never hyperpolarized but, again, slightly depolarized (less than 1 mV) by dibutyryl cyclic AMP (1 mM) in the presence of theophylline (2 mM). An example of this experiment is shown in Figure 1, a3 and b3.

The effect of dibutyryl cyclic AMP on the resting membrane potential of ganglion cells was also studied by recording the intracellular potential of individual ganglion cells. Detectable hyperpolarization was never observed in the presence of dibutyryl cyclic AMP (1 mM) in the absence or presence of theophylline (2 mM), a finding which is in agreement with the recent report by Kuba & Nishi (1976). However, it should be noted here that adrenaline-induced hyperpolarizations have not been recorded intracellularly in this particular preparation (Nakamura & Koketsu, 1972).

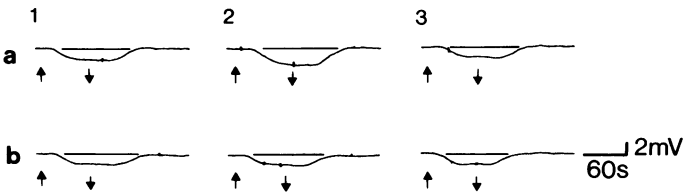

Figure 2 The effect of (a) adrenaline (0.3 mM) and (b) dibutyryl cyclic AMP (1 mM) on the K^+-activated hyperpolarization recorded from a sympathetic ganglion by the sucrose-gap method. (a and b) Records 1, and 2 were before and 20 min after application of drugs, respectively, and records 3 were 30 min after withdrawal of these drugs. These K^+-activated hyperpolarizations were produced by changing the perfusate from the K^+-free solution to the Ringer solution; the duration of applications of the Ringer solution is indicated by arrows. Note an augmentation of the K^+-activated hyperpolarization in the record a2, and its absence in record b2.

Effect of dibutyryl cyclic AMP on the K^+-activated hyperpolarization

It has been suggested that the slow i.p.s.p. and also adrenaline-induced hyperpolarization might be generated by an activation of the electrogenic Na^+ pump (Koketsu & Nishi, 1967; Nishi & Koketsu, 1967; 1968b; Nakamura & Koketsu, 1972; Koketsu & Nakamura, 1975; 1976). In connection with this suggestion, it was demonstrated (Akasu & Koketsu, 1976a,b) that adrenaline was able to potentiate the K^+-activated hyperpolarization which was generated by an activation of the electrogenic Na^+ pump (Rang & Ritchie, 1968; see also Akasu, Shirasawa & Koketsu, 1975). If cyclic AMP mediates such an action of adrenaline, it is to be expected that the amplitude of the K^+-activated hyperpolarization would be augmented in the presence of dibutyryl cyclic AMP. Experimental procedures for recording the K^+-activated hyperpolarization from the preparations and for examining the effect of a drug on this hyperpolarization by use of the sucrose-gap method were essentially similar to those described elsewhere (Akasu *et al.*, 1975; Akasu & Koketsu, 1976b). The augmentation of the K^+-activated hyperpolarization of ganglion cells was first confirmed in the presence of adrenaline (0.3 mM), then dibutyryl cyclic AMP was added after the withdrawal of adrenaline. The K^+-activated hyperpolarization recorded 20 min after an application of dibutyryl cyclic AMP showed no significant changes, as shown in Figure 2.

Effects of theophylline on the adrenaline-induced hyperpolarization

The adrenaline-induced hyperpolarizations of ganglion cells were recorded by use of the sucrose-gap method (cf. Nakamura & Koketsu, 1972). The effect of theophylline (2 mM) on these hyperpolarizations was examined. In 5 preparations, within approximately 15 min after addition of theophylline, the adrenaline-induced hyperpolarizations were never augmented. In 3 preparations they were depressed, as shown in record a3 of Figure 1.

Effects of theophylline on slow potentials

The P- and LN-potentials could be recorded from nicotine-treated or curarized ganglia by applying preganglionic nerve stimulation (30 Hz for 4 s) (Koketsu & Nishi, 1967; Nishi & Koketsu, 1967; 1968b). The effect of theophylline on these potentials was examined by use of the sucrose-gap method. In 11 preparations, within approximately 15 min after an addition of theophylline (2 mM) to the perfusate, the amplitude of P-potential and the LN-potential were depressed. These actions of theophylline were completely reversible; the amplitude of the P-potential and the LN-potential returned to the control value within approximately 30 min after theophylline was withdrawn. An example of these experiments is shown in Figure 3. The depression of LN-potentials by theophylline varied depending on individual ganglia. Thus, when the depression was strong, the initial part of LN-potentials even reversed potential polarity, as seen in Figure 3c. Such a unique change in the response (P- and LN-potentials) appeared to be explained by the change in the slow e.p.s.p. (see Discussion section).

In bullfrog sympathetic ganglia in which nicotinic transmission is completely blocked in the presence of nicotine (0.24 mM), or (+)-Tc (0.14 mM), the slow e.p.s.p. can be recorded easily with an intracellular electrode inserted into a cell-body of the ganglion cell (Nishi & Koketsu, 1968a; Tosaka, Chichibu & Libet, 1968; Kuba & Koketsu, 1974; 1976). Although the slow i.p.s.p., which precedes the slow e.p.s.p., can be recorded by an intracellular microelectrode from some cells, particularly from C-type cells (Tosaka *et al.*, 1968), the size of recorded slow i.p.s.p. is always very small (less than 2 mV) compared with the slow e.p.s.p. (cf. Figure 4b).

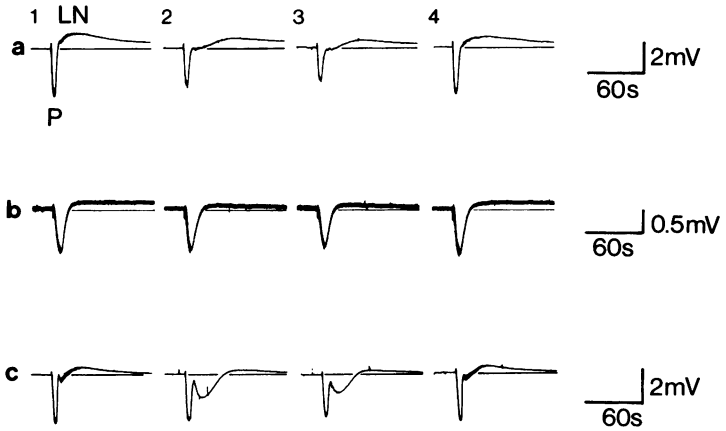

Figure 3 The effect of theophylline (2 mM) on the P- and LN-potential recorded from the nicotine-treated (a and c) and curarized (b) sympathetic ganglia. Records 1 were control, records 2 and 3 were obtained 15 min and 30 min after application of theophylline, respectively, and records 4 were approximately 30 min after its withdrawal. Note the depression of P- and LN-potentials in (a) and (b), and a unique change of the potential pattern in (c) (see text).

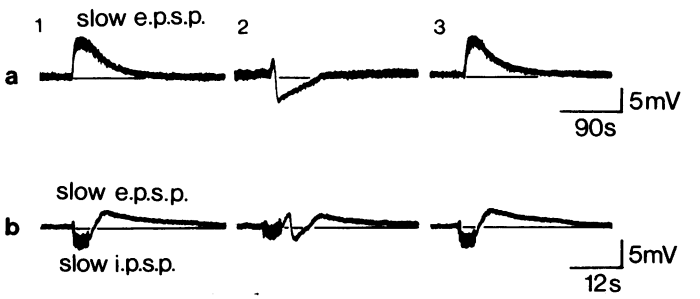

Figure 4 Effects of theophylline (2 mM) on the slow e.p.s.p. (a and b) and slow i.p.s.p. (b) recorded by intracellular microelectrodes from two different nicotine-treated sympathetic ganglion cells. These potential changes were evoked by preganglionic nerve stimulations (30 Hz for 4 s); the slow i.p.s.p. which precedes the slow e.p.s.p. is seen only in (b). (a and b) Records 1 and 2 were before and 15 min after application of theophylline, and records 3 were 30 min after its withdrawal. Note theophylline-induced hyperpolarizations triggered by the slow e.p.s.p. (a and b), and a depression of the slow i.p.s.p. (b) with theophylline.

When theophylline (2mM) was applied to nicotine-treated or curarized ganglia for more than approximately 10 min, the ganglion cell membrane was spontaneously and transiently hyperpolarized with a characteristic periodicity. These theophylline-induced hyperpolarizations were also observed in the absence of nicotine or (+)-Tc and were essentially similar to the caffeine-induced hyperpolarizations recently reported by Kuba & Nishi (1976). The slow e.p.s.p. recorded in the presence of theophylline was abolished by a transient large hyperpolarization. When the slow e.p.s.p. was generated by preganglionic stimulation, its initial depolarization triggered a large transient hyperpolarization which was similar to the spontaneously occurring hyperpolarization and ranged from 5 to 20 mV (Figure 4).

The size of the slow i.p.s.p. was never augmented but rather tended to be depressed in the presence of theophylline. These results were similar to those observed in the P-potential. An example of these results is shown in Figure 4b. These effects of theophylline on the ganglion cells were completely reversible; the hyperpolarization triggered by the slow e.p.s.p. and also the other theophylline-induced hyperpolarizations disappeared within approximately 30 min after theophylline was withdrawn. It should be

noted that these theophylline-induced hyperpolarizations were recorded, as in the case of caffeine-induced hyperpolarizations (Kuba & Nishi, 1976), from a limited proportion of cells.

Discussion

In the present experiments, dibutyryl cyclic AMP caused no hyperpolarization in the bullfrog sympathetic ganglia, in which the adrenaline-induced hyperpolarization was actually observed. Furthermore, theophylline did not potentiate but rather tended to depress the P-potentials in curarized or nicotine-treated ganglia, and depressed the adrenaline-induced hyperpolarization. None of these results support the hypothesis (McAfee & Greengard, 1972) that the P-potential (slow i.p.s.p.) as well as the adrenaline-induced hyperpolarization of sympathetic ganglia are produced by an increase of cyclic AMP in ganglion cells. Discrepancies between the results obtained from mammalian (McAfee & Greengard, 1972) and present preparations are difficult to explain satisfactorily at present, although they might be simply due to a difference between species.

A facilitating effect of dibutyryl cyclic AMP on the K^+-activated hyperpolarization was not observed in our experiments on the sympathetic ganglion cells in which the facilitating effect of adrenaline on the K^+-activated hyperpolarizations had been confirmed. This suggests that the action of adrenaline on the K^+-activated hyperpolarization may not be mediated by cyclic AMP. It should be noted here that the K^+-activated hyperpolarization of ganglion cells is considerably augmented in the presence of ATP (Akasu, 1976).

Although methylxanthines (e.g. caffeine, theophylline) are known to inhibit the enzyme activity of phosphodiesterase which catalyzes cyclic AMP, it is also known that these drugs release Ca^{2+} from sarcoplasmic reticulum into the myoplasm (Weber & Herz, 1968) and increase Ca^{2+} permeability of the myoplasmic membrane (Bianchi, 1961). Kuba & Nishi (1976) suggested that the caffeine-induced hyperpolarizations were the result of an increased conductance to K^+ which was probably caused by a rise in the intracellular Ca^{2+} concentration. In this way, the depression of LN-potential by theophylline was due to the generation of theophylline-induced hyperpolarizations. Indeed, the slow e.p.s.p. was abolished when these theophylline-induced hyperpolarizations were triggered by slow e.p.s.p., during which the intracellular Ca^{2+} concentration might be increased (Kuba & Koketsu, 1974; 1976). Since these hyperpolarizations are generated only from limited numbers of ganglion cells producing slow e.p.s.p., the degree of depression of the LN-potential would vary depending on individual ganglia. The pattern of LN-potential shown in Figure 3 appeared to be due to a large number of cells producing these hyperpolarizations triggered by slow e.p.s.p.

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