ACTION OF DIBUTYRYL CYCLIC ADENOSINE MONOPHOSPHATE ON THE INTESTINAL SMOOTH MUSCLE

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Cyclic adenosine 3', 5'-monophosphate (cyclic 3', 5'-AMP) is considered to be the secondary messenger of the beta-adrenergic action. Although exogenously applied cyclic 3', 5'-AMP does not gain access to the intracellular fluid in significant amount (1), the N6-2'-O-dibutyryl derivative, dibutyryl cyclic adenosine monophosphate (dibutyryl cyclic AMP) has a clear action, which is thought to be due to two properties of dibutyryl cyclic AMP, a possibly increased entry into cells and a greater resistance to hydrolysis. In this paper, effects of dibutyryl cyclic AMP were tested on the taenia caecum (or taenia coli) and the glycerated taenia caecum from the guinea pig to analyse its inhibitory action on the smooth muscle. The experimental results in this paper were selected from at least 7 experiments.

In order to examine the effects of dibutyryl cyclic AMP on the membrane, the experiments were made on strips of the taenia caecum from the guinea pig. A 10 mm in situ length of the taenia caecum was dissected free from underlying tissues and mounted in a sucrose gap apparatus (2). Locke Ringer solution gassed with a mixture of 95% O2 and 5% CO2 and kept at 32°C was used as bath fluid. Locke Ringer solution used in this paper contained 9.0 g of NaCl, 0.4 g of KCl, 0.2 g of CaCl2, 0.2 g of MgCl2, 0.5 g of NaHCO3 and 0.5 g of glucose in a litre. The membrane potential was amplified by a high gain d.c. amplifier and recorded on a pen oscillograph. Tension change was simultaneously recorded by using a mechano-electric transducer (RCA-5734). Drugs were given into 1 ml bath through which Locke Ringer solution constantly flowed.

Dibutyryl cyclic AMP (10^-4 g/ml) stopped the spontaneous spike discharge with some hyperpolarization (Fig. 1). These were accompanied by a tension decrease. The action of dibutyryl cyclic AMP was unaffected by 10^-6 g/ml of propranolol which was enough concentration to block the action of isoprenaline (Fig. 1).

To test the effect of dibutyryl cyclic AMP on the contractile elements of the smooth muscle, the glycerated taenia caecum was used. A glycerated muscle was prepared by the method originally described by Bozler (3). A piece of the taenia caecum, after washing in 2 mm MgCl2 for 30 minutes, was stored in 35% glycerol solution kept at -15 to -18°C for several weeks. This glycerol solution also contains 60 mm KCl, 15 mm Tris and 4 mm MgCl2. The response of the glycerated muscle suspended in 2 ml organ bath

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FIG. 1. Actions of dibutyryl cyclic AMP and isoprenaline on the electrical and mechanical activities of the taenia caecum and effect of propranolol on the actions of the both drugs. upper trace : electrical activity, lower trace : mechanical activity.

A-1, B-1; control responses, A-2, B-2; in the presence of propranolol (10^{-5} g/ml) DiBu.C-AMP : dibutyryl cyclic AMP. concentration shown : final concentration.

filled with a solution containing 80 mm KCl, 20 mm Tris and 4 mm MgCl_2 adjusted to pH 6.8 by maleic acid and kept at 25°C was isometrically recorded. The glycerated taenia caecum prepared by this method responded to adenosine triphosphate (ATP) alone. No further increase in tension was observed by an addition of Ca ions (2 mm). However, increased tension was decreased by 2 mm ethylenedioxybis(ethyleneamino)tetraacetic acid (EGTA). This indicates that the glycerated taenia caecum contains Ca ions in itself. The 3 minutes pretreatment of dibutyryl cyclic AMP (3 × 10^{-4} g/ml) was without any effect on tension of the preparation induced by ATP (4 mm) alone. Furthermore, tension produced by ATP (4 mm) was not decreased by addition of dibutyryl cyclic AMP (3 × 10^{-4} g/ml) (Fig. 2 upper). The following procedure was made to abolish the endogenous Ca ions in the muscle. Another piece of the taenia caecum, which was washed in 2 mm MgCl_2, was also stored in 35% glycerol solution kept at -15 to -18°C for several weeks. The glycerol solution contains 60 mm KCl, 15 mm Tris and 4 mm EGTA. The glycerated taenia caecum was suspended in 2 ml organ bath filled with a solution containing 80 mm KCl, 20 mm Tris and 4 mm EGTA adjusted to pH 6.8 by maleic acid and kept at 25°C. Tension was also isometrically recorded. Tension of the glycerated muscle was increased
FIG. 2. Effect of dibutyryl cyclic AMP on tension of the glycerated taenia caecum.

upper: on the muscle stored in 35% glycerol solution containing 60 mm KCl,
15 mm Tris and 4 mM MgCl₂.

lower: on the muscle stored in 35% glycerol solution containing 60 mm KCl,
15 mm Tris and 4 mM EGTA.

diBu.C-AMP: dibutyryl cyclic AMP.

concentration shown: final concentration.

by 4 mM ATP containing 4 mM MgCl₂ (ATP-Mg) and 5.0 x 10⁻⁷ M Ca ions. The concentration of Ca ions was adjusted by varying the ratio of total Ca and EGTA and was calculated as described by Imai and Takeda (4). Tension of the glycerated muscle induced by ATP-Mg and Ca ions was not decreased by dibutyryl cyclic AMP (3 x 10⁻⁴ g/ml) (Fig. 2 lower). These indicate that dibutyryl cyclic AMP does not inhibit the function of the contractile elements but suppresses the membrane activity of the smooth muscle. Therefore, the inhibitory action of dibutyryl cyclic AMP and perhaps cyclic 3', 5'-AMP seem to be concerned with the inhibition of supply of Ca ions to the contractile elements, which is due to suppression of the membrane activity.

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