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Spasmolytic action of adenosine on the guinea-pig ileum

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Adenosine (10^{-5}M) reduces the contractile response of the guinea-pig isolated ileum to 5-hydroxytryptamine or barium more than the responses to acetylcholine or histamine. Adenosine also inhibits the contractile response of the guinea-pig ileum to a calcium-free medium. These results suggest that adenosine effects a blockade of the indirect responses to barium or 5-hydroxytryptamine mediated through intrinsic nerves and has a weak direct inhibitory effect on the smooth muscle cells of this tissue.

Smooth muscle is generally relaxed by adenosine (Bennet & Drury, 1931), although certain smooth muscle structures, such as the kidney vasculature (Buyniski & Rapela, 1969) are contracted. Adenosine produces coronary vasodilatation (Rubio & Berne, 1969), relaxation of bronchioles (Titone, 1914), and decreases spontaneous activity of cat and rabbit intestinal musculature (Drury & Szent-Gyorgyi, 1929).

We have found that, using guinea-pig ileum, indirect-acting agonists are blocked more effectively by adenosine than direct-acting agonists. Thus, in certain smooth muscles, the relaxant effect of adenosine and the antagonism of indirect-acting drugs by adenosine may be due in part to an action on the intrinsic nerve supply.

METHODS

Female guinea-pigs, 400 to 800 g, were killed by cervical dislocation; sections of ileum were removed and Tyrode solution passed through the lumen. Strips of ileum, 4 to 5 cm in length were fixed in a 70 ml bath and attached to a Grass Force Displacement transducer (Ft. 03). The normal bathing fluid was Tyrode solution having a composition (mM): NaCl, 136; KCl, 2·7; $MgCl_2$, 1·4; NaH_3PO_4, 0·04; $CaCl_2$, 1·8; $NaHCO_3$, 11; and glucose, 5·5 (pH = 7·5). Air was vigorously bubbled into the baths which were maintained at 37°. One g of tension was placed on each muscle strip and the change in isometric tension was recorded on a Grass Polygraph.

A 30 min equilibration period was allowed before addition of drugs, which were added directly to the muscle bath in volumes that did not dilute the bath more than 2%. Each intestinal strip was exposed to only one agonist, after each addition of which approximately 20 s was allowed before washing out with Tyrode. The maximum tension reached in the period of contraction was recorded.

Doses of acetylcholine, histamine, 5-hydroxytryptamine (5-HT) and barium required to produce 40% of the maximum response (ED40) were determined from dose response curves (Saferna, Loukomskaya & others, 1966). These doses were repeatedly added to, and washed from, the solution bathing the ileal preparations at 5 min intervals for a total of 105 min. Adenosine was added 20 min after the first

response was obtained, and was removed 45 min later. Control experiments without adenosine, involving at least two muscle strips for each agonist, showed that the responses to the ED40 increased slightly at first, reaching a relatively stable value in 20 to 30 min. No correction was made for the initial increase in response noted in control experiments.

In another series of experiments, dose-response curves were done for each agonist before and after addition of adenosine and after removal of adenosine. Adenosine remained in contact with the ilea for 30 min before addition of agonist. The recovery dose-response curve was obtained 30 min after washing out adenosine. The muscles were washed once after each dose of agonist and at least three times between each dose response curve.

RESULTS

Antagonism of the response to acetylcholine by adenosine. The response of the guinea-pig ileum to the ED40 of acetylcholine was decreased by adenosine, 10^{-5}M, an average of 38% in six experiments. The decrease was significant at the 1% level by comparison of the means and standard deviations of the three control responses before adenosine with the first three responses after addition of adenosine (Fig. 1A). However, the dose response curve to acetylcholine was not significantly affected by 10^{-5}M (Fig. 2A). Reduction in the responses to the ED40 appears to be a more

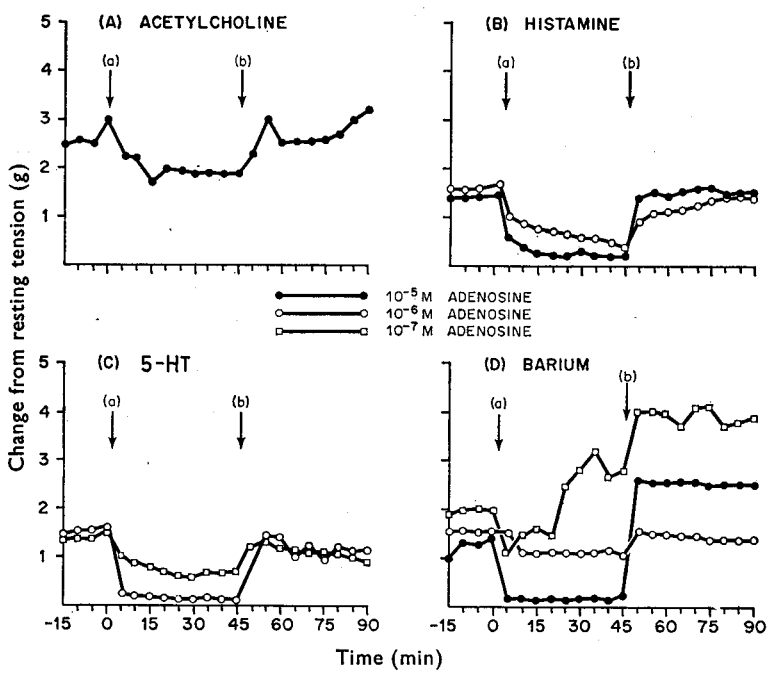

FIG. 1. Antagonism by adenosine of the responses of the guinea-pig ileum to various agonists. A dose of each agonist needed to produce 40% of the maximal response was repeatedly added to, and washed from, the medium bathing the ilea. The maximum response obtained in 20 s was recorded. Adenosine was added at (a) and was washed out at (b). Doses of agonist averaged $7{\cdot}2 \times 10^{-8}$M for acetylcholine, $3{\cdot}6 \times 10^{-7}$M for histamine, $5{\cdot}4 \times 10^{-8}$M for 5-HT, and $8{\cdot}75 \times 10^{-5}$M for barium. Each point is a mean of 5 to 6 experiments, except for barium with 10^{-7}M adenosine where 3 muscles were used.

sensitive measure of the effect of an antagonist. It is concluded that adenosine 10^{-5}M, weakly antagonizes the response of the guinea-pig ileum to acetylcholine.

Antagonism of the response to histamine by adenosine. Fig. 1B shows that the response to the ED40 of histamine is strongly reduced (90%) by 10^{-5}M adenosine ($P < 0{\cdot}01$). Again the effect is not so apparent in the dose-response to the agonist (Fig. 2B), although the responses to lower doses of histamine were significantly antagonized ($P < 0{\cdot}01$, $P < 0{\cdot}01$ and $P < 0{\cdot}02$ respectively for 0·39, 1·3, and 3·9 $\times$ 10^{-7}M histamine). Higher doses of histamine were not significantly affected by adenosine, 10^{-5}M.

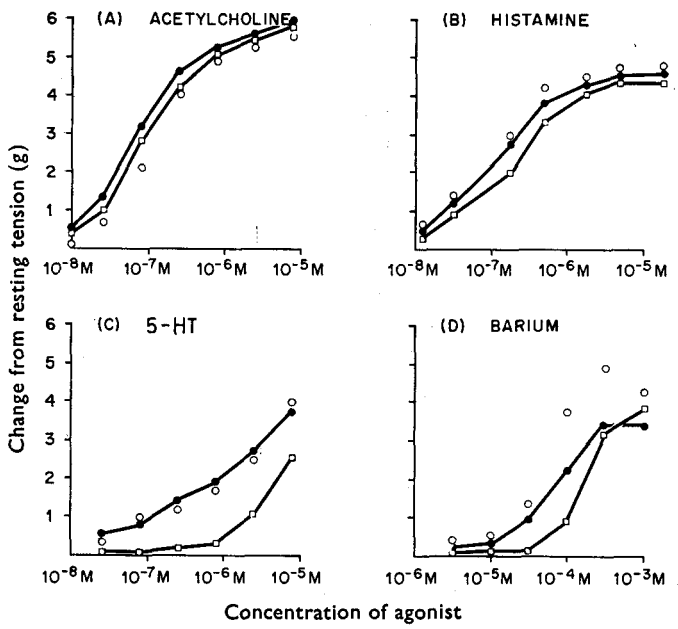

FIG. 2. Dose-response curves to various agonists before (●), during (□), and after (○) exposure to adenosine, 10^{-5}M. Each point is a mean of 8 to 12 experiments.

Antagonism of the response to 5-hydroxytryptamine by adenosine. A nearly complete blockade of the response to the ED40 of 5-HT was seen in the presence of 10^{-6}M adenosine (Fig. 1C). After addition of adenosine the first response of 5-HT was significantly less than the control response before adenosine ($P < 0{\cdot}01$ at 10^{-7}M adenosine and $P < 0{\cdot}001$ at 10^{-6}M adenosine). Also, the dose-response curve to 5-HT was markedly depressed by 10^{-5}M adenosine (Fig. 2C). When the reciprocal of the dose of 5-HT is plotted against the reciprocal of the mean response, straight lines are obtained for the responses in the presence and in the absence of adenosine. The slopes of the lines are significantly different ($P < 0{\cdot}02$) by regression analysis which suggests that this antagonism is competitive.

Antagonism of the response to barium by adenosine. The response to the ED40 of barium was nearly abolished by 10^{-5}M adenosine (Fig. 1D). At lower concentrations of adenosine, antagonism was not marked. The data suggest that adenosine in low concentrations may enhance the response to the ED40 of barium. After exposure for 20 min to 10^{-7}M adenosine the muscles showed an exaggerated response to barium

rather than an antagonism. Also, on removal of adenosine (10^{-7}, 10^{-6}, and 10^{-5}M), the responses to barium were higher on the average than control responses before adenosine and the difference bordered on significance ($P < 0{\cdot}06$). In the dose-response curves to barium, lower doses were markedly antagonized by 10^{-5}M adenosine (Fig. 2D). The nature of the antagonism was obscured by the enhancement above control of the response to barium at the highest dose ($P < 0{\cdot}01$).

Effect of adenosine on the contractile response of guinea-pig ileum to calcium-free medium. Exposure of guinea-pig ileum to a calcium-free medium (containing 5×10^{-6}M Na_2EDTA) after soaking in normal Tyrode solution produces a contraction of 1 to 2 g tension lasting approximately 3 min (30 observations). This type of contraction in guinea-pig ileum is thought to be independent of any intrinsic nerve supply since it is not blocked by procaine or atropine (Irwin & Oliver, 1970). Adenosine, 10^{-5}M, inhibits the contractile response to a calcium-free medium when added before removal of calcium (30 observations) and abolishes the contraction when added after the response to a calcium-free medium has been initiated (12 observations).

DISCUSSION

Adenosine strongly antagonized the response of the guinea-pig ileum to 5-HT or barium whereas the antagonism to acetylcholine was only weak. Antagonism of the response to histamine by adenosine was also not marked, especially in the dose response curves. The actions of 5-HT and barium are mediated to a large extent through the intrinsic nerve supply, whereas acetylcholine and histamine are thought to act directly on the smooth muscle of guinea-pig ileum (Gershon, 1967; Paton & Zar, 1968; Henderson, Ariëns & Simonis, 1970). These data suggest that a major part of the inhibitory action of adenosine is exerted on the intrinsic nerve supply of guinea-pig ileum.

These observations are in accord with the report that adenosine inhibits intrinsic intestinal reflexes (Bishop, Frazer & others, 1963). Both the preparatory and emptying phases of the response of the guinea-pig ileum to distention are completely blocked by adenosine. Thus, adenosine blocks reflexes mediated through the intrinsic nerves of guinea-pig ileum, and also blocks the actions of drugs mediated through this system.

Enhancement by adenosine of the response of the guinea-pig ileum to large doses of barium has not been reported previously. The mechanism by which the increased response is produced is not known. Blockade by adenosine of the release of an inhibitory substance by barium is a possible explanation.

A relatively weak blockade by adenosine of the actions of acetylcholine and histamine on guinea-pig ileum was seen. These agonists act directly on this tissue to produce the contractile response. It was also shown that adenosine blocks the contractile response of guinea-pig ileum to a calcium-free solution. This response is also thought to be independent of any intrinsic nerve supply in the isolated ileum (Irwin & Oliver, 1970). Thus, it appears that the relaxant effect of adenosine in the guinea-pig ileum is mediated through the intrinsic nerve supply and to a lesser extent through a direct action on smooth muscle cells.

Cocaine is also a more effective antagonist of the effect of 5-HT on the guinea-pig ileum than of the effects of either acetylcholine or histamine (Roche e Silva, Valle & Picarelli, 1953). This suggests that adenosine may act as a local anaesthetic in

guinea-pig ileum, but intradermal injection of 0·1 ml of 4×10^{-3}M adenosine into guinea-pigs does not alter the response to an electrical stimulus applied to the skin at the injection site making it unlikely that a local anaesthetic action explains the spasmolytic effects of adenosine.

Since adenosine occurs physiologically (Douglas, 1966; Rubio & Berne, 1969), it is possible that variation in its endogenous levels may give rise to variation in the action of certain drugs on the gastrointestinal tract.

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