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SHORT COMMUNICATIONS

Desensitization of ionophore A23187 responses by muscarinic receptor stimulation in intestinal smooth muscle

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The divalent cation selective ionophore A23187 has been shown to initiate a wide variety of Ca^{2+}-dependent events [1, 2]. It is widely accepted that A23187 serves as an electroneutral Ca^{2+} carrier to dissipate Ca^{+2} gradients across plasma and intracellular membranes [3, 4]. Several observations indicate, however, that the actions of A23187 are more complex [2]. A dependence upon Na^{+}_{EXT}, as well as upon Ca^{2+}_{EXT}, has been shown for A23187-induced deplorization of pancreatic acinar cells [5] and for contractions of guinea pig ileal smooth muscle [6]. Additionally, A23187 responses in several systems, including guinea pig ileum, are sensitive to the Ca^{2+} channel antagonists D600 and Nifedipine employed at concentrations that inhibit known Ca^{2+} channel processes [2, 6–9]. The insensitivity of A23187 responses in guinea pig ileum to tetrodotoxin, atropine, mepyramine, and other antagonists indicates that its effects are not mediated through transmitter release [6, 10]. The responses to A23187 and to muscarinic agonists, however, are very similar in their Ca^{2+}_{EXT} dependence, Na^{+}_{EXT} dependence, and sensitivity to Ca^{2+} channel antagonists, thus indicating that both agents may initiate a common event—activation of voltage-sensitive Ca^{2+} channels. In this communication, we describe an additional common aspect of muscarinic agonist and A23187 action whereby a muscarinic agonist produces desensitization in the guinea pig ileal smooth muscle to A23187.

Strips of guinea pig ileal longitudinal smooth muscle were prepared according to a previously described method [6] for recording isotonic responses in Tyrode's solution of the following composition (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCL_2$, 0.88; NaH_2PO_4, 0.36; $NaHCO_3$, 12.0; and dextrose, 5.5. Tissues were initially equilibrated in this solution for 1 hr, with a solution change every 15 min. A control response to a just supramaximal concentration (5×10^{-7} M) of the selective and potent muscarinic agonist [11] *cis*-2-methyl-4-dimethyl-aminomethyl-1,3-dioxolane methiodide (CD) was determined and, following a further hour of equilibration and washing, the responses to submaximally effective concentrations A23187 (8×10^{-7} M), CD (8×10^{-8} M), or K^+ (60 mM) were determined and expressed as percentages of the fast component of the CD response (Fig. 1a).

In desensitization experiments, tissues were treated with CD (5×10^{-7} M) for 10 min. The tissues were then relaxed by washing for a further 10 min, and the response to A23187 (8×10^{-7} M), CD (4×10^{-8} M), or K^+ (60 mM) was determined. These concentrations of A23187 and CD gave responses that were approximately 60 per cent of that produced by CD (Fig. 1a). After densitization these responses were reduced to ~20 per cent of control (Fig. 1b). The reverse of this experiment, pretreatment with A23187 and determination of CD response, could not be accomplished because of the difficulty in reversing A23187 responses.

Since the responses to both CD and A23187 are dependent upon Ca^{2+}_{EXT}, it is possible that receptor stimulation depletes a pool of Ca^{2+} critical to the maintenance of mechanical response. This is unlikely because K^+ responses were not affected by the prior treatment with CD (Fig. 1b) although K^+ and CD responses are equally dependent upon Ca^{2+}_{EXT} and are equally sensitive to La^{3+}, D600 and Nifedipine [11, 12], consistent with their using a common pool of Ca^{2+}.

Although responses of guinea pig ileum to CD and A23187 show many similarities, it is unlikely that the desensitization of A23187 responses by prior muscarinic receptor stimulation represents an interaction between the ionophore and the muscarinic receptor, for this desensitization is also directed against other agonists including histamine, substance P, and 5-hydroxytryptamine ([13, 14]; K. Jim and D. J. Triggle, unpublished results). Rather, the interaction must be sought at a post-receptor site. Several possibilities may be considered. Ca^{2+} channel inactivation following prolonged agonist exposure may be responsible for densensitization [15, 16], but this is unlikely, for during

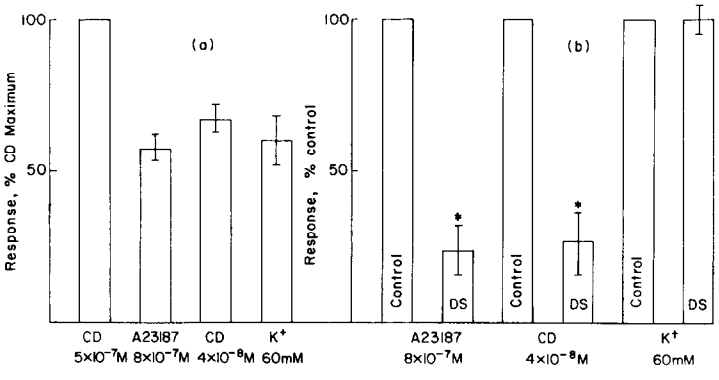

Fig. 1. Panel (a): Graphical representation of maximum response produced by CD (5×10^{-7} M), and submaximum responses produced by A23187 (8×10^{-7} M), CD (4×10^{-8} M), and K^+ (60 mM). Between determination of each response, tissues were washed for 60 min. The vertical bars indicate S.E.M.; N = 6. Panel (b): Desensitization produced by exposure of tissue to CD (5×10^{-7} M) for 10 min followed by wash for 10 min. The responses to the submaximum concentrations of A23187, CD, and K^+ are normalized to 100 per cent (control), and the responses to these same concentrations are shown following prior CD exposure (DS). The responses to A23187 and CD are both significantly reduced (*) by the desensitizing procedures ($P < 0.001$; N = 6).

the 10-min desensitizing exposure to CD no reduction in mechanical response was seen. Furthermore, no desensitization is seen after K^+ exposure (K. Jim and D. J. Triggle, unpublished results) although K^+ and CD responses are equally sensitive to Ca^{2+} channel antagonists [11]. More plausibly, the hyperpolarization mediated by electrogenic Na^+ pumping following removal of a muscarinic agonist in the ileal longitudinal muscle [17, 18] reduces the activity of depolarizing agonists [19]. This suggestion is consistent with our previously reported suggestion that A23187 action in this tissue involves a Na^+-dependent depolarization mediating activation of voltage sensitive Ca^{2+} channels [6]. In any event, the densensitization of A23187 responses by prior muscarinic receptor activation is not consistent with A23187 serving as a simple Ca^{2+} carrier.

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Effect of alcohol on hepatic secretion of methylfolate ($CH_3H_4PteGlu_1$) into bile*

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Acute alcohol ingestion is associated with a rapid fall in serum folate level that, in part, may relate to an alcohol effect on the folate enterohepatic cycle. Past experiments with an alcoholic rat model showed a marked decrease in biliary folate matched by increased formation of hepatic polyglutamate [1]. Previously, Brown *et al.* [2] had reported a reduction in liver polyglutamate formation in alcoholic rat liver. Lane *et al.* [3] postulated a blocking effect of alcohol on mobilization and transport of folate from tissue to plasma in alcoholic man. The latter study employed the flushing technique of Johns *et al.* [4] to measure the rate of tissue storage and utilization of $^{14}CH_3H_4PteGlu_1$.

To further investigate the different results obtained in these studies, we examined the effect of the flushing technique on release of isotopic folate from liver in the alcoholic rat model. The tendency for an increased conversion to polyglutamate was again demonstrated. However, a simultaneous resistance to flush of labeled folate from the alcoholic hepatocyte was also apparent, suggesting an alcohol-induced defect in the transport of folate into bile.

Female Sprague–Dawley rats weighing 150–250 g were used. Three groups of animals were compared: (1) normal animals maintained on a standard Purina rat chow diet containing 30 μg/g of *Lactobacillus casei* active folate; (2) folate/nutrient deprived (F/ND) animals maintained by feeding tube for 3 days on a liquid diet of 25% sucrose in water to which 100 mg of succinyl sulfathiazol/100 ml was added to suppress intestinal production of folate; and (3) folate/nutrient deprived alcoholic animals (F/ND–EtOH) maintained by feeding tube on 100 ml/kg per day of a solution of 10% ethanol for 3 days, together with the succinyl sulfathiazol/sugar water diet. After 3 days, bile duct cannulation was performed as described previously [1]. While still under anesthesia, 100 ng of [^{3}H]$PteGlu_1$ (sp. act. 20 Ci/mmole) was injected by tail vein. All bile was then collected for the next hour, including 20 μl samples at 10-min intervals for isotopic counting. At the end of 1 hr, five control animals from each dietary group were killed; the livers were removed, weighed, and immediately prepared for counting and chromatography [5]. An additional five control animals from each group were killed after 2 hr for chromatographic analysis of liver folate.

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