Short communications

An effect of calcium on histamine desensitization of guinea-pig ileum

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Desensitization of guinea-pig ileum by histamine was significantly decreased in the presence of a high concentration of calcium in the desensitizing solution.

It is a common observation that the administration of a large dose of histamine or acetylcholine in the isolated guinea-pig ileum is followed by a period of desensitization, during which the responses to the desensitizing drug are reduced. Desensitization by histamine of the guinea-pig ileum is partly unspecific, in that the responses to other agonists may also be reduced (Cantoni & Eastman, 1946 ; Paton, 1961) and partly selective for histamine (Schild, 1973), but in this communication only the desensitizing effect of histamine towards histamine itself is examined. It will be shown that the concentration of calcium in the Ringer solution during the desensitization phase influences the degree of desensitization.

Methods.—Responses of the isolated guinea-pig ileum to histamine were recorded isometrically, using automatic assay apparatus and automatic syringes for injecting drugs. A previously described servo-apparatus (Schild & Seaford, 1970) was employed which automatically re-adjusted the initial tension of muscle to a predetermined value (0·4 g) and could also record length changes. The ileum was suspended in Tyrode solution at 30° C, containing (mM) NaCl, 137 ; KCl, 2·7 ; $NaHCO_3$, 12 ; NaH_2PO_4, 0·5 ; glucose, 5·5 ; $CaCl_2$, 2, except that during the desensitization period when solutions of varying calcium content were applied, HEPES Ringer, at 30° C, containing (mM) NaCl, 147 ; glucose, 5·5 ; HEPES buffer (Wellcome) 5 ; $CaCl_2$, 0·2–4 ; bubbled with O_2 ; (pH 7·7) was used since HEPES (*N*-2 hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid) does not bind calcium appreciably (Good, Winget, Winter, Connolly, Izawa & Singh, 1966). Differences in tonicity due to differing calcium contents of solutions were compensated with sodium chloride. A fixed routine was adopted as follows: 1. Period of stabilization in 2 mM Ca-Tyrode. Dose-response curves were established with 4, 8, 16 ng/ml histamine (final bath concentrations). 2. Desensitization in HEPES Ringer containing 0·2–4·0 mM Ca. Immediately after immersion in HEPES Ringer, a standard desensitizing dose of histamine (0·4 μg/ml final bath concentration) was applied, causing maximal isotonic shortening. The desensitizing histamine solution was left in contact for exactly 10 minutes. 3. Brief recovery period (3 min) in Ca-HEPES Ringer without histamine. Muscle relaxed. 4. Further recovery period in 2 mM Ca-Tyrode solution during which dose-ratios were determined at approximately 3 min intervals. Dose-ratios were calculated by interpolation from the earlier histamine dose-response curves. Two strips from the same intestine were generally used with contrasting calcium concentrations during the desensitization phase. Frequently a second desensitization assay was performed on the same piece after recovery. Otherwise new preparations from different animals were used in each experiment.

Results.—Two independent sets of experiments are illustrated in Figure 1. Figure 1A shows the time course of desensitization after application of the desensitizing histamine solution in 0·2 and 4·0 mM Ca^{++}. The post-desensitization dose-ratios are markedly different in the two cases, being less after high calcium, the difference persisting for at least 30 minutes. Figure 1B shows the relation between calcium concentration in the desensitizing histamine solution and dose-ratio at a constant time (10 min) after desensitization. Concentrations of 0·2, 0·5, 1·0, 2·0 and 4·0 mM Ca^{++} were used. A symmetrical design was adopted in which each calcium concentration occurred four times and each contrast twice. The log dose-ratios showed a monotonic decline with increasing calcium concentration. The differences in log dose-ratios were highly significant by the variance ratio test ($F=18·5$, $P<0·001$).

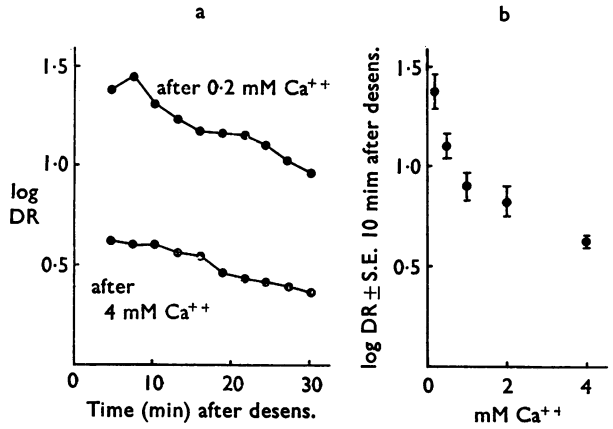

FIG. 1. Histamine dose-ratios after a desensitizing dose of 0·4 μg/ml histamine was applied for 10 minutes. The desensitizing histamine dose was administered in HEPES Ringer containing various Ca^{++} concentrations; otherwise Tyrode (2 mM Ca^{++}) was used. Contractions of guinea-pig ileum recorded isometrically. (a) Time course of desensitization. Log dose-ratios after applying desensitizing dose in 0·2 and 4·0 mM Ca^{++} (n=4). Zero time represents the moment at which the desensitizing dose was washed out. (b) Calcium dependence of desensitization. Log dose-ratios and S.E. (n=4) 10 min after washing out desensitizing dose in 0·2, 0·5, 1·0, 2·0 and 4·0 mM Ca^{++}.

Discussion. — Post-desensitization dose-ratios are composite functions depending on the degree of preceding desensitization and the rate of recovery (Rang & Ritter, 1970) but in the present comparative experiments the short-term dose-ratios probably reflect mainly the relative degree of preceding desensitization since the recovery rates were uniformly slow. The important factor determining the degree of desensitization seemed to be the concentration of external calcium during the time of application of the desensitizing histamine dose.

The effect of calcium on histamine desensitization of guinea-pig ileum is interesting, if only because of our general ignorance of the mechanism of drug desensitization, but it is as yet unknown whether this is an isolated phenomenon, confined to histamine acting on guinea-pig ileum or whether it has wider significance. It would be interesting to know whether calcium affects desensitization by other drugs, and whether other ions, including sodium, potassium and magnesium interact with calcium in respect of desensitization.

The effect of calcium could be interpreted in several different ways. For example calcium could 'protect' the receptors against desensitization by histamine, or alternatively, histamine desensitization could be related to the exhaustion of a calcium store required for the action of histamine. The latter explanation is in accordance with the general hypothesis (Durbin & Jenkinson, 1961; Edman & Schild, 1962; Daniel, 1965; Van Breemen & Lesser, 1971) that drugs which stimulate smooth muscle do so by raising the level of free intracellular calcium, either liberating calcium from a bound site, or increasing permeability to extracellular calcium. A desensitization mechanism by way of exhaustion of a calcium store seems attractive but would not, alone, fully account for the present findings. It fails to explain why the desensitizing effect of a large histamine dose should persist even after readmitting Tyrode solution, whose high calcium content might be expected to replenish the calcium stores.

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