0.2 ± 0.03 °C ($p < 0.005$). The larger individual variation in the response of the interscapular site resulted in the increase of 0.2 ± 0.10 °C being nonsignificant ($p > 0.1$). Corresponding values for the large areas over the neck, shoulder and interscapular site were 0.4 ± 0.04 ($p < 0.001$), 0.3 ± 0.07 ($p < 0.025$) and 0.2 ± 0.10 °C (NS, $p > 0.1$) respectively.

Following this initial increase in temperature at 30 min, a slight decrease was next observed and this was followed by a sustained rise in temperature which reached a peak at approximately 1.5 h (fig.2). For all 3 sites this rise represented a significant increase above the baseline value. For the neck, interscapular region and shoulder the mean temperature differences ± SEM from time 0, for the small areas, were 0.4 ± 0.11 ($p < 0.025$), 0.3 ± 0.09 ($p < 0.05$) and 0.4 ± 0.11 ($p < 0.025$) °C. Corresponding values for the 3 large areas were 0.3 ± 0.08 ($p < 0.05$), 0.3 ± 0.07 ($p < 0.025$) and 0.4 ± 0.09 ($p < 0.010$) °C respectively.

After this 2nd peak at approximately 1.5 h the skin temperatures gradually returned to the baseline values so that by 2.5 h the mean differences ± SEM were -0.05 ± 0.18 ($p > 0.5$), -0.15 ± 0.11 ($p > 0.2$) and -0.08 ± 0.09 ($p > 0.4$) °C for the small areas of the neck, interscapular site and shoulder respectively.

Thus, 2 peaks in skin temperature were observed to follow the meal. It could be speculated that the 1st rapid increase was due to the stimulation of brown adipose tissue, since the increase in circulating noradrenaline occurs immediately after a meal. And, that the 2nd sustained rise was the result of the general vasodilatation which occurs when the heat produced on processing and oxidation of food is only gradually lost from the body[9]. However, the postulated explanation for the 1st increase in temperature seems unlikely, since there was no preferential increase in skin temperature over the suspected areas of brown fat. After a meal, the increase in skin temperature over areas of the back where brown adipose tissue is thought to exist was no greater than over areas where no evidence has been reported for the presence of this tissue. Unless brown fat also exists on the shoulder, it must be concluded that the rises in skin temperature over the neck, interscapular region and shoulder which occurred after a meal, must have been due to increased blood flow and general vasodilatation. It is conceivable, however, that brown adipose tissue in the deep body sites was activated by the meal and that IR-thermography was unable to detect any such activation. In addition, the possibilities that changes which could be firmly attributed to brown adipose tissue occur after either a very large meal or prolonged overfeeding in man have not been eliminated[10].

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Desensitization to histamine in the absence of external Ca^{++} in the guinea-pig taenia caecum

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Summary. Short-term desensitization of the contractile response of the guinea-pig taenia caecum to histamine was tested in the absence of Ca^{++}. Desensitization was monitored both by the fall of histamine response and by the decrease of irreversible blockade by phenoxybenzamine. In Ca^{++}-free solution with 0.2 mM EGTA, desensitization occurred as in normal physiological solution containing Ca^{++}.

Short-term desensitization to histamine was first observed early by Barson and Gaddum[1]. Intestinal smooth muscle loses its sensitivity to histamine on contact exposure to histamine itself. In skeletal and cardiac muscle, Ca^{++} plays a critical role in desensitization of cholinergic receptors[2,3]. Thus it is essential to study the influence of Ca^{++} on desensitization of smooth muscle. As the contraction of the guinea-pig taenia caecum in response to histamine is dependent solely on the influx of Ca^{++} [4], desensitization to this agonist should provide information on demand for Ca^{++}. Kenakin and Cook[5] reported that after desensitization of the histamine H_1 response, phenoxybenzamine (POB) became less effective in irreversible blockade of histamine response than in non-desensitized muscle, indicating transient change of affinity of the receptor system. We used this method in addition to direct measurement of the change of response after desensitization to histamine.

Materials and methods. Male guinea-pigs, weighing 250-400 g, were killed instantly by cervical fracture and exsanguination. Strips of taenia caecum were suspended in a 30-ml organ bath bubbled with air at 30 °C. The bathing solution was Locke-Ringer solution composed of 154 mM NaCl, 5.63 mM KCl, 2.10 mM $CaCl_2$, 2.10 mM $MgCl_2$, 5.95 mM $NaHCO_3$ and 5.55 mM glucose (normal solution). In Ca^{++}-free solution, $CaCl_2$ was omitted from the normal solution and 0.2 mM EGTA (glycol ether diaminetetraacetic acid) was added. Cumulative contractile response was recorded isotonically with a lever of circa 0.5 g load on a smoked drum. For desensitization in Ca^{++}-free solution, after responses in the normal solution were observed, the

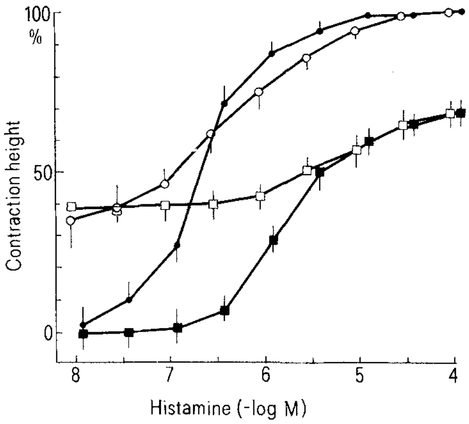

Figure 1. Desensitization to histamine in the absence of external Ca^{++} (open symbols) and in the presence of Ca^{++} (filled symbols) in the guinea-pig taenia caecum. Isotonic contractions to cumulative doses of histamine are expressed in percent of the maximal response in Ca^{++}-containing solution before any treatment. Desensitization in the absence of Ca^{++} was induced by incubating the muscle in Ca^{++}-free Locke-Ringer solution containing 0.2 mM EGTA for 30 min in the presence of 10^{-4} M histamine. Then Ca^{++}-containing Locke-Ringer solution was reintroduced and the response to histamine was measured after 20 min (□). Controls (○) were treated similarly, but without 10^{-4} M histamine. Note that the tone of the preparation was increased after exposure to Ca^{++}-free solution (see Methods). For desensitization in the presence of Ca^{++}, Ca^{++}-containing solution was used throughout the experiments: desensitized muscles (■) and controls (●). All points in this figure are the means ± SE of 6 experiments.

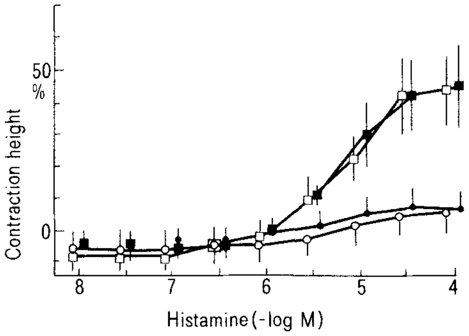

Figure 2. Irreversible blockade by phenoxybenzamine (POB) of histamine-induced contraction before and after desensitization in the absence (open symbols) or in the presence (filled symbols) of external Ca^{++} in the guinea-pig taenia caecum. The recording conditions and desensitization procedure were the same as in figure 1. After desensitization or control treatment in Ca^{++}-free Locke-Ringer solution, POB (5×10^{-6} M) was added for 20 min. The preparation was then returned to Ca^{++}-containing solution and the responses to histamine (in percent of the maximal response to histamine in normal solution before POB treatment) were repeatedly measured until the response reached plateau (2–3 h (□). Controls (○) were treated similarly, but without 10^{-4} M histamine. For desensitization in the presence of Ca^{++}, Ca^{++}-containing Locke-Ringer solution was used throughout the experiments: desensitized muscles (■) and controls (●). Note that the tone of the preparation was decreased after POB treatment (see methods section). All points in this figure are the means ± SE of 5 experiments.

muscle was washed four times with Ca^{++}-free solution, soaked in Ca^{++}-free solution and washed twice with Ca^{++}-free solution with 10-min interval between washings. Desensitization was induced in Ca^{++}-free solution by incubating the muscle with 10^{-4} M histamine for 30 min. Then the muscle was washed twice with Ca^{++}-free solution and the response was determined in normal solution 20 min after the end of the incubation. For desensitization in the presence of Ca^{++}, normal solution was used throughout the experiment. Other conditions were the same as above. For experiments with POB in Ca^{++}-free solution, the muscle was washed twice with Ca^{++}-free solution after desensitization in Ca^{++}-free solution as above and POB was added in Ca^{++}-free solution 10 min after the end of incubation for desensitization. After incubation for 20 min with POB, the muscle was washed with Ca^{++}-free solution and soaked in normal solution. Responses were recorded in normal solution after repeated washings (2–3 h). Each control was treated similarly, but without 10^{-4} M histamine. All the contractile responses were expressed as the percentages of the maximal response to histamine in normal solution before any treatment. Changes in tone of the preparation caused by Ca^{++}-free treatment or by phenoxybenzamine application were also expressed in percent of the same maximal control response. The statistical significance of differences was evaluated by Student's t-test and $p = 0.05$ was taken as the upper limit of significance.

Results and discussion. Lack of external Ca^{++} had no effect on desensitization of the histamine H_1 response in the guinea-pig taenia caecum. In Ca^{++}-free solution with 0.2 mM EGTA, desensitization of the response to histamine occurred as shown in figure 1. The base lines of the dose-response curves in the case of desensitization in Ca^{++}-free conditions were raised, probably by sudden change of the bathing solution from Ca^{++}-free solution to normal one. But the maximal contractile response of the desensitized muscle was significantly less than that of the non-desensitized one as was the case after desensitization in normal solution. We tested the transient change in affinity of the receptor system by measuring the irreversible blockade with POB. POB irreversibly blocked histamine response in Ca^{++}-free solution in the same degree as in normal solution. As shown in figure 2, even in Ca^{++}-free solution POB was significantly less effective in the desensitized muscle than in the non-desensitized one, indicating that the affinity was reduced. Thus, desensitization in meaning both loss of response and loss of affinity occurred even in the absence of external Ca^{++}. In the Ca^{++}-free solution no response to histamine was observed and the influx of Ca^{++} induced by histamine should be very small. Contraction per se and massive influx of Ca^{++} are not the cause of desensitization. However, Casteels and Raeymaekers[6] reported that in the absence of external Ca^{++} histamine might still mobilize Ca^{++} from an intracellular store and it might be possible that intracellular Ca^{++}, whose amount is not sufficient to cause any contraction, is involved in the desensitization mechanism. We are now studying the relationship of such Ca^{++} to desensitization in smooth muscle.

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