Short communications

Dependence of β-adrenoceptor mediated mechanical effects on the glycogen concentration in the rabbit isolated small intestine

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In the rabbit isolated intestine, tachyphylaxis was evident in the inhibitory responses to isoprenaline, but not in those to phenylephrine, ATP or dibutyryl cyclic AMP. The tachyphylaxis to isoprenaline was associated with a spontaneous fall in the tissue glycogen concentration and was enhanced when the glycogen concentration was markedly reduced by incubation of the tissue under anaerobic conditions in glucose-free Krebs solution. Doubling the glucose concentration of the Krebs solution slowly restored the response to isoprenaline and prevented the development of tachyphylaxis to it. The results support the concept that the β-adrenoceptor mediated response is dependent on some product of cellular metabolism.

Isoprenaline, phenylephrine, adenosine triphosphate (ATP) and the dibutyryl analogue of cyclic adenosine 3′,5′ monophosphate (dibutyryl cyclic AMP), acting through different mechanisms, all inhibit the pendular movements of the rabbit small intestine (Bowman & Hall, 1970). In the present experiments some observations on the relationship between inhibition of pendular movements by these drugs and tissue glycogen concentration were made.

Methods.—Isolated segments of small intestine from Dutch strain rabbits of either sex were suspended in Krebs solution (NaCl 118 mM, KCl 4·7 mM, $CaCl_2$ 2·5 mM, $MgCl_2$ 1·2 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1·1 mM, glucose 5·6 mM) at 37° C and gassed with 5% carbon dioxide in oxygen. Pendular movements were recorded on a kymograph as described in a previous paper (Bowman & Hall, 1970). Glycogen concentrations in segments of intestine were measured by the method described by Montgomery (1957).

Glycogen depletion in the guinea-pig taenia coli is hastened if the tissue is exposed to substrate-free Krebs solution, first anaerobically and then aerobically (Bueding & Hawkins, 1964). Segments of rabbit intestine were therefore treated in this way. They were first incubated in glucose-free Krebs bubbled with 5% carbon dioxide in nitrogen for 1 h and then for a further hour while bubbled with 5% carbon dioxide in oxygen.

Results.—When repeated additions of the same concentration of phenylephrine hydrochloride (0·598–1·196 μM; 0·12–0·24 μg/ml), ATP (2·0–5·0 μM; 1·1–2·75 μg/ml) or dibutyryl cyclic AMP (8–10 mM; 3·93–4·91 mg/ml) were made to the intestine, their inhibitory effects on pendular movements remained approximately constant over a period of several hours. However, when isoprenaline bitartrate (0·047–0·094 μM; 16·6–33·2 ng/ml) was added in the same way, the inhibitory response gradually diminished in size over a period of several hours, so that after about 2 h, the response was reduced by 50–75% of that at the start of the experiment.

It was not necessary to make repeated additions of isoprenaline in order to observe a dimunition of response, although repeated applications enhanced the rate of fall off. When the isolated intestine was left in oxygenated Krebs solution, a second addition of the same, or of a freshly prepared isoprenaline solution, made 2–3 h after the first, produced a greatly diminished response whereas a second addition of phenylephrine, ATP or dibutyryl cyclic AMP produced a response comparable to the first. Fig. 1a illustrates the results of experiments in which glycogen levels were estimated immediately after removal of the intestine from the animals, and again, 2 h later, at a time when the response to isoprenaline, but not that to phenylephrine, had diminished. There was a spontaneous fall in the mean (±S.E.) glycogen concentration of 32·0±10·2% ($P<0·02$) which was associated with the diminished responses to isoprenaline.

Figure 1b illustrates the results of experiments on intestinal segments in which glycogen depletion had been hastened by first aerobic followed by anaerobic incubation in glucose-free Krebs solution. The mean (±S.E.) glycogen concentrations fell to

25·6±3·9% ($P<0·001$) of the mean control concentrations at the start of the experiment and this was associated with abolition of the response to the standard concentration of isoprenaline. The spontaneous pendular movements were unaltered by this treatment and the inhibitory responses to phenylephrine (Fig. 1b), ATP and dibutyryl cyclic AMP remained similar to the controls. Incubation in glucose-free Krebs solution, but under aerobic conditions, also enhanced the depletion of glycogen, but to a lesser extent than that occurring under anaerobic conditions. After 2 h incubation in glucose-free Krebs solution bubbled with 5% carbon dioxide in oxygen, glycogen concentrations had fallen to a mean (±S.E.) of 61·7±5·5% ($P<0·005$) of the control concentrations at the start of the experiments.

Responses to isoprenaline after glycogen depletion were not restored by the addition of glucose (5·6 mM), glycogen (0·4–0·8 mg/ml), glucose-6-phosphate (1–10 mM), glucose-1-phosphate (1–10 mM), pyruvate (0·8–40 mM), lactate (10–40 mM), succinate (10–30 mM) or β-hydroxybuty-

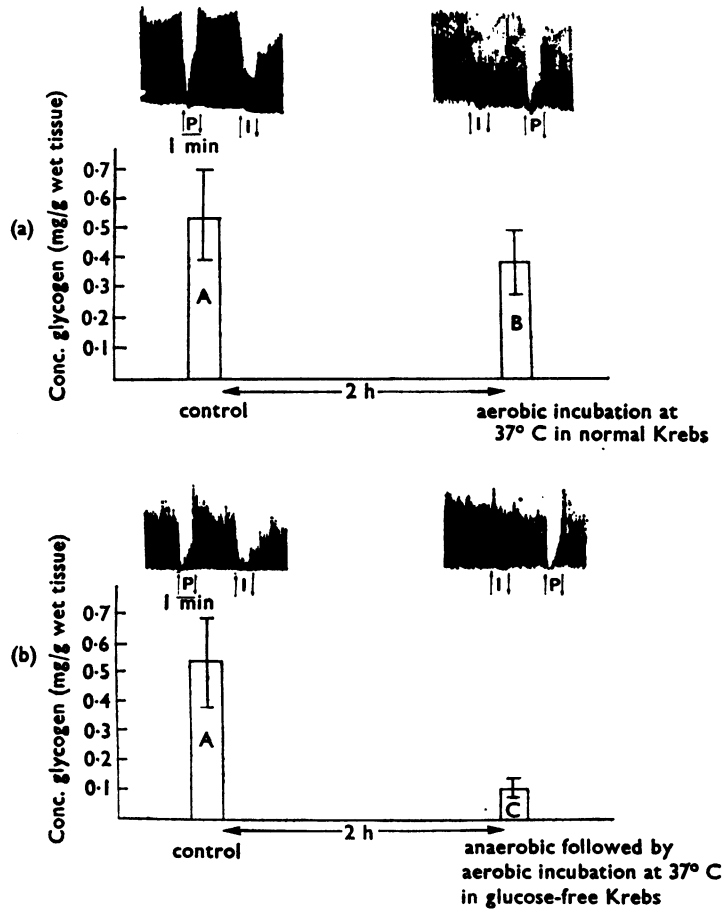

FIG. 1. (a), Segments of small intestine from 11 rabbits were assayed for glycogen on removal from the animals (A). In each case, the segments were suspended in the organ bath and their responses to drugs tested. The records shown are typical of the responses to phenylephrine at P(0·598 μM; 0·1 μg/ml) and isoprenaline at I(0·047 μM; 10 ng/ml). Two hours after incubation in oxygenated Krebs at 37° C the response to isoprenaline was diminished and at this time the segments were assayed for glycogen (B). (b), Similar experiments except that the segments of intestine were incubated first anaerobically and then aerobically in glucose-free Krebs at 37° C to hasten glycogen depletion (C). The variation in the day to day control levels of glycogen are reflected in the large standard errors of this figure.

rate (10–30 mM). The lack of effect of some of these substances may have been due to their inability to penetrate cell membranes. Although the response to isoprenaline did not reappear in normal Krebs solution, doubling the glucose concentration gradually caused its reappearance, so that 60 min after the glucose concentration was doubled, the response to isoprenaline had returned to 75–90% of its control size. Tachyphylaxis to isoprenaline developed much more slowly, or not at all, in tissues that were suspended in Krebs solution containing double the normal concentration of glucose, from the start of the experiment.

Pronounced changes in pH are known to modify responses to isoprenaline in some tissues (Bygdeman, 1963; Reynolds & Haugaard, 1967) but this was not found to be a factor in the present experiments, because no change in pH could be detected.

Discussion.—The characters of the inhibitory responses to α- and β-adrenoceptor agonists in the rabbit small intestine differ. α-Adrenoceptor agonists, such as phenylephrine, produce an inhibition of rapid onset from which recovery occurs despite the continued presence of the drug. On washout, there is usually an overshoot in contraction height. β-Adrenoceptor agonists, such as isoprenaline, produce an inhibition of slower onset, that is maintained throughout the presence of the drug, and there is no overshoot on washout. ATP produces a response similar in character to that produced by phenylephrine, whereas the response to dibutyryl cyclic AMP resembles that to isoprenaline (Bowman & Hall, 1970). The present experiments demonstrate a further difference between the responses to α- and β-adrenoceptor agonists. Tachyphylaxis is evident in the responses to the β-adrenoceptor agonist, isoprenaline, but not to the α-adrenoceptor agonist phenylephrine. Tachyphylaxis to isoprenaline was associated with a spontaneous diminution in tissue glycogen concentration and appeared to be a consequence of this, since the reduction in the response to isoprenaline was enhanced when the rate of reduction of tissue glycogen concentration was hastened by anaerobic incubation in glucose-free Krebs solution. Andersson and Mohme-Lundholm (1969, 1970) also found the response of the rabbit colon and taenia coli to isoprenaline to be dependent on the tissue glycogen concentration.

These results indicate that the response to β-adrenoceptor agonists is dependent on some product of cellular metabolism. Cyclic AMP is a likely candidate for this role in view of the work of Sutherland and his co-workers (Robison, Butcher & Sutherland, 1968) and the observation that dibutyryl cyclic AMP remains effective after glycogen depletion, is compatible with this.

It is possible that β-adrenoceptor responses in general are dependent on the maintenance of tissue glycogen stores. At a late stage following shock due to endotoxin (Bhagat, Cavanagh, Merrild, Rana & Rao, 1970; Parratt, unpublished observations) and haemorrhagic shock (Page, 1944; Walton, Richardson, Walton & Thomson, 1959; Wyse & Beck, 1971) the heart becomes less responsive to the actions of catecholamines and sympathetic nerve stimulation. The hypoxic conditions induced by these types of shock probably deplete the cardiac glycogen stores and the ineffectiveness of the sympathetic nerves and catecholamines may be a consequence of this. Measures designed to restore the cardiac glycogen stores might therefore contribute to effective treatment.

I should like to thank Professor W. C. Bowman for his advice in the writing of this manuscript.

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(*Received March* 3, 1972)