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Selective activity of cyclokinins

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Based upon physicochemical data and theoretical calculations it has been suggested that, in solution, kinins such as bradykinin take on a quasi-cyclic configuration in which an ionic bond is formed between the guanidyl group of arginine 1 and the arginylcarboxyl at position 9 (Ivanov et al 1975; Galaktinov et al 1977). More recent spectroscopic data provides no evidence for such cyclic structures of kinins (London et al 1978) yet Chipens et al (1981) suggest they do occur in the hydrophobic receptor biophase. Indeed the latter group have synthesized cyclokinins in which ring closure was imposed by covalent bond formation and have reported potent vasoactive properties (Chipens et al 1981).

Recently we have shown that kinins are extraordinarily potent stimulants of chloride secretion in the mammalian colon (Cuthbert & Margolius 1982) and have obtained small amounts of two cyclokinins for a limited pharmacological study on ion transport processes. The synthetic kinins we have used are ε-cyclo[Lys^1-Glys^6]-bradykinin (cBK) and ε-cyclokallidin (cK) (UCB, Bioproducts Peptide Dept., Brussels). Their structures are compared with that of kallidin (lysylbradykinin, LBK) below

Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH (Kallidin)

Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (cK)

Lys-Pro-Pro-Gly-Phe-Gly-Pro-Phe-Arg (cBK)

Experiments were made on epithelia dissected from the descending colon of male Sprague-Dawley rats. The epithelia were mounted in Ussing-type chambers (window 0·6 cm^2) and were voltage clamped at zero potential (short circuited) while bathed in Krebs-Henseleit solution at 37 °C and gassed with 95% O_2 – 5% CO_2. The method is given in more detail elsewhere (Cuthbert & Margolius 1982).

Kallidin and cyclokinins were added to the serosal bath. The increases in short circuit current (SCC), which in the case of LBK has been shown to be due to an increase in chloride secretion (Cuthbert & Margolius 1982), have been converted to μequiv of monovalent anion secreted by 0·6 cm^2 in 10 min in the data presented in Fig. 1. Both of the cyclokinins produced small increases in SCC at a concentration of approximately 10 μM which were equivalent to those produced

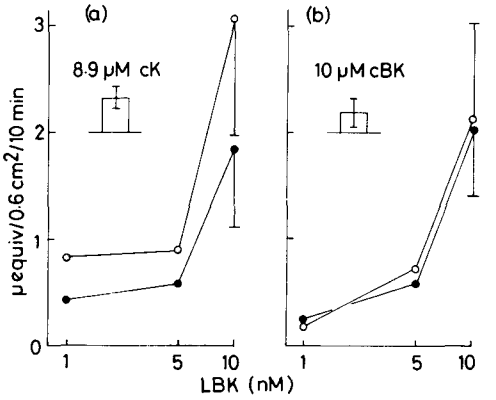

FIG. 1. Paired preparations of rat colon epithelia were prepared for recording and allowed to stabilize for about 30 min. cK (8·9 μM) or cBk (10 μM) was added to the serosal bathing fluid of one preparation and its effect on SCC recorded. The area under the curve for the 10 min following peptide addition was converted to μequiv. The mean responses (± S.E.) to the cyclokinins are shown as insets in (a) and (b). Partial concentration-response curves to LBK were obtained in the presence (●) and absence (○) of the cyclokinins. Standard errors are shown for LBK at a concentration of 10 nM. The responses were significantly different ($P < 0·05$, using paired t-test) with cK but not with cBK. Number of paired observations was 5 in (a) and 6 in (b).

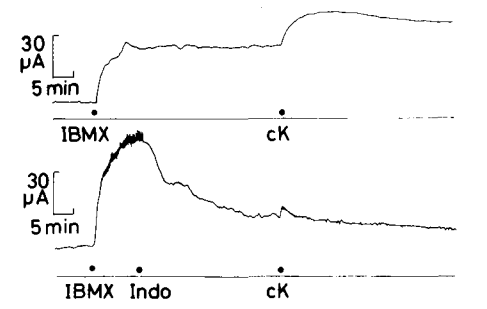

FIG. 2. SCC records from two paired preparations. Both preparations were treated with IBMX (0.1 mM) and the preparation represented by the lower tracing was also treated with indomethacin (1 μM). Cyclokallidin (4·45 μM) was then added to the serosal bathing fluid of each preparation. Note that a response is obtained only in the absence of indomethacin. The horizontal lines indicate zero SCC.

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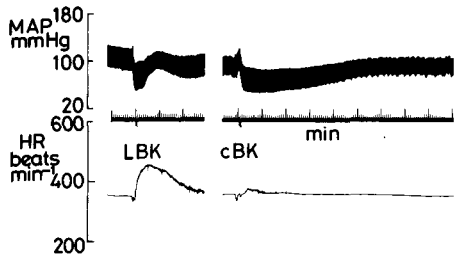

FIG. 3. Comparison of the responses to LBK and cBK, both at 100 nmol kg^{-1}, in the anaesthetized rat. The effects of the kinins on arterial pressure and on heart rate are shown.

by 10 000 times less LBK. We had insufficient material to obtain concentration-response curves to the cyclokinins, but measured partial concentration-response curves to LBK in the presence of approximately 10 μM cyclokinins. We found that cK but not cBK produced a significant reduction in the responses to LBK at 10 nM. Thus cK appears to behave as a weak partial agonist and may be a useful lead compound for kinin antagonists, however the nature of the antagonism by cK is not known and even making a number of assumptions its affinity is unlikely to be greater than 10^5 M^{-1}.

Thus far we have shown that cK causes a minor effect on SCC but not necessarily that this effect is kinin-like. Kinin effects on the colon are known to be prostaglandin-dependent and abolished by indomethacin (Cuthbert & Margolius 1982) and furthermore the effects of kinins are potentiated by inhibition of phosphodiesterase with isobutylmethylxanthine (IBMX) (Cuthbert et al 1982). Consequently we designed experiments in which paired preparations were treated with IBMX (0·1 mM) with or without indomethacin (1 μM). Subsequent addition of cyclokinins (approximately 5 μM) to both preparations produced a response only in the preparation untreated with indomethacin. Fig. 2 shows an example of this type of experiment for cK and shows that the actions of cyclokinins, like LBK, appear to be dependent on prostaglandin formation.

Therefore it is found that the effects of cyclokinins are disappointingly weak and that cK has a weak antagonistic action against LBK on chloride secretion in the colon. As potent vasodepressor activity was reported for the cyclokinins (Chipens et al 1981) we repeated and extended the earlier experiments on blood pressure in the anaesthetized rat. Male Sprague-Dawley rats were anaesthetized with urethane (5 ml kg^{-1}, 25% w/v solution by intraperitoneal injection). Body temperature was maintained at 37 °C by use of a heating pad. Blood pressure was recorded from the right carotid artery by a pressure transducer and heart rate (HR) was recorded by a ratemeter triggered by the pulse pressure. Both parameters were displayed continuously and drugs were given by intravenous injection into the left jugular vein in volumes of less than 0·1 ml per 100 g and washed in with 0·2 ml 0·9% NaCl (saline). The mean arterial pressure (MAP) is here defined as the diastolic pressure plus one third of the pulse pressure. In addition we measured the duration of the responses, defined as the time for the mean arterial pressure to return to its original value.

Fig. 3 shows responses to LBK and cBK at equivalent concentrations. Immediately apparent is the prolonged action of the cyclokinin compared with the linear analogue. The results of a series of experiments in which

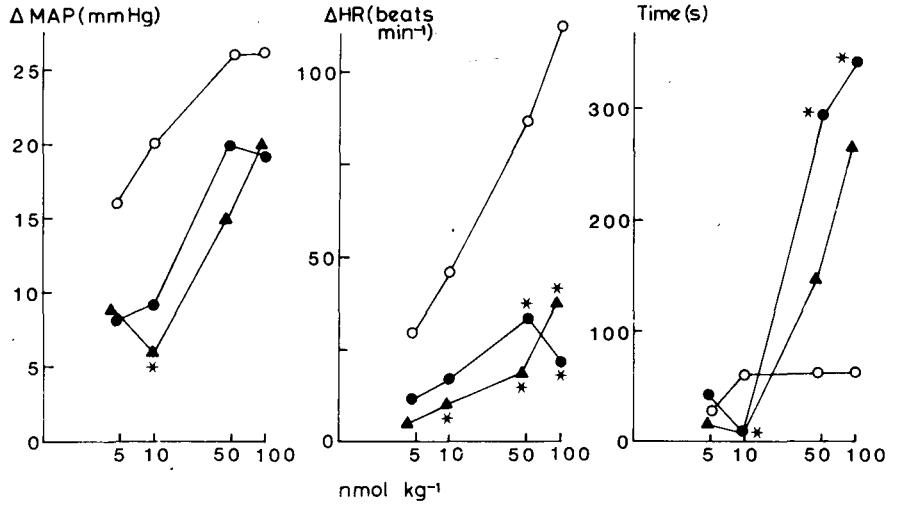

FIG. 4. Dose response curves to LBK (○), cBK (●) and cK (▲) in the anaesthetized rat. The reduction in mean arterial pressure (ΔMAP) is shown at the left, the increase in heart rate (ΔHR) in the middle and the duration of the response is shown at the right. Of the 36 values shown in this figure 28 are the means of 4 observations. Due to lack of material the other 8 values were means of 2 or 3 observations. Where points are marked with an asterisk the value is statistically different from the corresponding value for LBK.

a range of doses of kallidin and the two cyclokinins were compared is given in Fig. 4. Comparable falls in MAP were produced by LBK and the cyclokinins, and although the mean values were lower for cK and cBK no significantly statistical difference could be established with the limited amount of material available. Both cyclokinins produced significantly less effect on HR compared to LBK. Kinins are known to cause the release of catecholamines from the adrenal medulla (Staszewska-Barczak & Vane 1967) which must help to terminate the vasodepressor action and increase HR. The cyclokinins are some five times less potent at increasing HR compared to LBK while the effect on MAP is, at most, only two-fold less. Chipens et al (1981) commented particularly on the prolonged vasodepressor activity caused by cBK, although they showed only single responses. In their hands bradykinin (50 nmol kg^{-1}) had a duration of action of 50 s, much the same as seen here for the same dose of LBK. The same dose of cBK in their hands produced a blood pressure fall lasting for several hours. We have not been able to confirm this although responses to cBK were of some six times greater duration than those to LBK. As we mentioned above, part of the difference in the duration of the responses to the linear and cyclic peptides may be related to differential ability to affect HR, and presumably vasomotor tone. There was no significant change in the ΔMAP responses to LBK after rats had been given indomethacin, 10 mg kg^{-1}, suggesting that the effects of kinins on blood pressure are not associated with prostaglandin formation.

Clearly the potency of the cyclokinins compared with LBK is very different when blood pressure responses or chloride secretion are measured. This may point to the existence of more than one type of receptor (see, for example, Regoli & Barabé 1980) or a single type of receptor with different mechanisms. The ability of the cyclokinins to affect blood pressure is relatively unimpaired by the constraints imposed by the closed ring configuration. This does not, in our view, either support or contradict the hypothesis that kinins adopt a quasi-cyclic configuration in their interaction with the receptor. Clearly such restraint has significant consequences upon the interactions with epithelial receptors.

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