The nature of the bradykinin inactivating system in isolated lungs

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Bradykinin is extensively inactivated in the pulmonary circulation *in vivo* and in isolated lungs (for references, see Bakhle & Vane, 1974). Some of this inactivation is undoubtedly due to the action of a dipeptidyl carboxypeptidase (converting enzyme, kininase II, EC.3.4.15.1) which hydrolyses both bradykinin and angiotensin I. However, there are other peptidases (amino-, endo- or carboxy-) capable of inactivating bradykinin, as the cleavage of any peptide bond causes 99% biological inactivation (Suzuki, Abiko, Endo, Kameyama, Sasaki & Nabeshima, 1969) and we have tried to assess the contribution of peptidases other than converting enzyme to the overall inactivation of bradykinin in isolated perfused lungs by studying the fate of two analogues of bradykinin in this system.

The analogues used were 7-β homo Pro-bradykinin (7 HBK) and 8-β homo Phe-bradykinin (8 HBK) (Ondetti & Engel, 1975) and their inactivation in rat and guinea-pig isolated lungs was measured by bioassay using either the guinea-pig ileum or the cat terminal ileum (Alabaster & Bakhle, 1972). The results given are the means of at least six preparations. In rat lungs all the peptides were extensively inactivated and very little activity survived a single passage through the pulmonary circulation—bradykinin, 2.8 ± 0.8%; 7 HBK 10.6 ± 1.6%; 8 HBK 3.8 ± 1.2%. However, in guinea-pig lungs, whereas only a tenth of bradykinin (9.5 ± 1.0%) and 8 HBK (10.0 ± 1.2%) activity survived passage through the pulmonary circulation, nearly half of the activity of infusions of 7 HBK (42.0 ± 6.0%) emerged from the lung. The *Bothrops* nonapeptide, Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, inhibits the pulmonary inactivation of bradykinin (Greene, Camargo, Krieger, Stewart & Ferreira, 1972; Engel, Schaeffer, Gold & Rubin, 1972) by its inhibition of converting enzyme (Bakhle, 1974; Dorer, Kahn, Lentz, Levine & Skeggs, 1974; Das & Soffer, 1975). Infusion of the nonapeptide (100 ng/ml) through the pulmonary circulation protected bradykinin and 8 HBK from inactivation in guinea-pig lung, the percent survival increasing to 58.4 ± 4.1 and 55.8± 4.4 respectively whereas the inactivation of 7 HBK was not significantly affected. In rat lung the survival of 7 HBK was again unaffected by the nonapeptide whereas that of bradykinin and 8 HBK was increased to 12.2 ± 1.2% and 13.6 ± 2.8% respectively.

As the inactivation of 7 HBK is relatively resistant to the nonapeptide inhibitor we conclude that this analogue is not a substrate for converting enzyme, whereas 8 HBK and bradykinin are both substrates. This is compatible with the finding that 7 HBK was a more potent depressor agent than 8 HBK *in vivo* and whereas the hypotensive actions of 8 HBK and bradykinin were potentiated, that of 7 HBK was not (Ondetti & Engel, 1975). Our results also show that in isolated guinea-pig lung almost half of the observed bradykininase activity is converting enzyme-like, whereas in rat lung most of the bradykininase activity is not converting enzyme-like. This may explain results suggesting that the conversion of angiotensin I in rat lung is less than in the lungs of other species (Bakhle, Reynard & Vane, 1969; Kreye & Gross, 1971).

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Effects of some fatty acids on gallbladder function

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Prostaglandins are known to have actions both on fluid transport and on smooth muscle of the gallbladder (Morton, Saverymuttu & Wood, 1974). We have now investigated the effects of other fatty acids on gallbladder function.

In vitro experiments with intact gallbladders taken from male guinea-pigs showed that arachidonic acid, a known prostaglandin precursor, inhibited fluid transport, in a dose-dependent manner, within 30 min of serosal application in the concentration range 10-200 μM. Inhibition was preceded by a transient phase of enhanced fluid loss that was concurrent with a rise in intraluminal pressure; these last two effects probably being related (Morton, Saverymuttu & Wood, 1975).

Since these actions of arachidonic acid are seen with somewhat lower concentrations of prostaglandins of the E or F series (Morton, *et al.*, 1974) the possibility of conversion must be considered. Indomethacin (1 μM) was shown to antagonize the actions of arachidonic acid but, in contrast, to have little effect on those of ricinoleic acid, a fatty acid not thought to be converted to prostaglandin, although found here to have properties and potency similar to arachidonic acid when tested on the gallbladder.

These experiments suggest that, although arachidonic acid and ricinoleic acid have similar actions on gallbladder fluid transport and smooth muscle, the former owes some of its potency to conversion to prostaglandin. This is in agreement with the fining that the spasmogenic action of arachidonic acid on gallbladder strips is antagonized by indomethacin (Andersson, Hedner & Persson, 1974).

In view of similarities in the transport systems, these findings may also throw light on gastrointestinal function. It is well known, for instance, that ricinoleic acid has a pronounced cathartic action, although it is uncertain whether this is due to effects on fluid transport or on smooth muscle (Fingl, 1970).

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