Selective inhibition by mepacrine of the release of "rabbit aorta contracting substance" evoked by the administration of bradykinin

Intra-arterial injection of bradykinin or of arachidonic acid to guinea-pig isolated lungs is followed by the appearance in the effluent of "rabbit aorta contracting substance" (RCS). Formation of RCS is blocked by nonsteroidal acidic anti-inflammatory drugs (Piper & Vane, 1969; Vargaftig & Dao Hai, 1971) and by various sulfhydryl and antioxidant agents (Vargaftig & Dao Hai, 1972). RCS has been distinguished from prostaglandin (PG) $F_2\alpha$ and E_2, and indirect evidence suggests that their cyclic peroxide precursor is related with the activity of RCS (Gryglewski & Vane, 1971). Activation of an acylhydrolase is a necessary requirement for the triggering of the chain of events that ultimately leads to PG synthesis, except when the fatty acid precursor, as arachidonic acid, is directly provided, thus shortcutting the reaction. Evidence that the triggering enzyme is phospholipase A (phosphatide acylhydrolase, E.C.3.1.1.4.) has been summarized by Kunze & Vogt (1970).

We have tested the hypothesis that bradykinin and arachidonic acid act at different critical points of the chain of reactions leading ultimately to RCS formation: evidence that the anti-inflammatory and antimalarial agent mepacrine blocks the release of RCS by bradykinin, but is ineffective when arachidonic acid is used, is provided as follows.

Release of RCS from isolated perfused guinea-pig lungs was tested as previously described (Vargaftig & Dao Hai, 1971) by a modification of the method of Piper & Vane (1969). Guinea-pig lungs were perfused with Krebs solution containing antagonists to noradrenaline, 5-hydroxytryptamine and acetylcholine (phenoxybenzamine, 10^{-7}; methysergide and atropine, 10^{-6} g litre^{-1}), at a flow of 10 ml/min. The effluent superfused a cascade of isolated organs: rabbit aorta and pulmonary artery, to detect RCS, and a strip of rat stomach, to detect prostaglandins. Organ contractions and bronchial pressure were recorded through appropriate transducers on a Grass No. 7 Polygraph. The following drugs were used: arachidonic acid (Mann Research Labs); bradykinin and methysergide (Sandoz); phenoxybenzamine (Smith Kline & French) and $PGF_2\alpha$ (provided by Dr. A. van Dorp, Unilever, The Netherlands); mepacrine dihydrochloride (Rhone Poulenc).

Arachidonic acid (10–20 μg) was injected through the pulmonary artery into five isolated guinea-pig lungs. This was followed by the appearance in the perfusate of RCS activity, which was unaffected by a 10 min perfusion of the lung with Krebs solution containing mepacrine (20 μg/ml), whereas the RCS activity which followed the injection of 10 μg of bradykinin into five guinea-pig lungs was completely blocked. A thirty min perfusion with mepacrine-free Krebs solution resulted in complete recovery for the release of RCS by bradykinin (Fig. 1).

To ascertain the site of action, mepacrine was directly superfused upon the organ cascade, by-passing the lungs. Under such conditions, RCS released by bradykinin was clearly unaffected, showing that blockade occurs at the pulmonary level.

Suppression by mepacrine of the release of RCS when evoked by bradykinin but not when evoked by arachidonic acid is the first example of such a selective inhibition reported up to now. RCS is a prostaglandin precursor, probably the cyclic peroxide (Gryglewski & Vane, 1971) which requires the activation of an acylhydrolase, as phospholipase A, to be set free (Kunze & Vogt, 1970). Administration of arachidonic acid shortcuts the enzyme activation, directly providing the recognized substrate for $PGF_2\alpha$ and for PGE_2 formation (Anggard & Samuelsson, 1965). Mepacrine displays

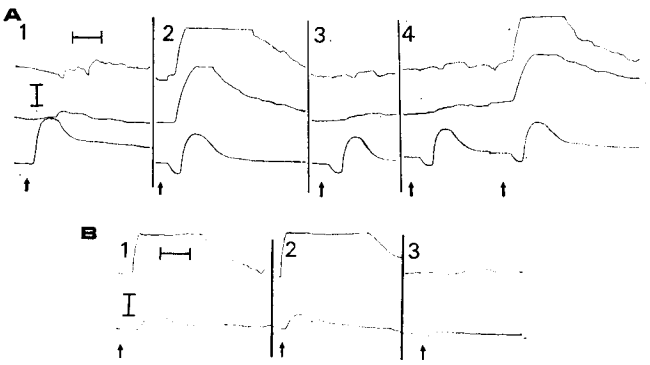

FIG. 1. A. Inhibition by mepacrine of the release of RCS by bradykinin. Responses of strips of rabbit aorta (upper tracing) and pulmonary artery (middle) and of rat stomach strip (lower) to the perfusate of an isolated guinea-pig lung. Arrows indicate administration of 10 μg of bradykinin, as follows: Panel 1, superfused directly upon the organs. Panel 2, injected into the lungs. Panel 3, injected into the lungs, after a 10 min perfusion with Krebs solution containing 20 μg/ml of mepacrine, thus blocking the release of RCS. Panel 4, the first response is to bradykinin directly superfused upon the organs, as in panel 1; the second response is to bradykinin injected into the lungs, thus showing the return of release of RCS. The organs were superfused with mepacrine throughout, at similar concentrations as those infused into the lungs thus discarding effects of the antagonist upon the organs. Horizontal scale represents 2 min; vertical scale represents 2 cm.

B. Absence of inhibition by mepacrine of the release of RCS by arachidonic acid. Responses of strips of rabbit aorta (upper tracing) and of rat colon (lower) to the perfusate of a guinea-pig isolated lung. Arrow indicate administration of 20 μg of arachidonic acid as follows: Panel 1, injected to the lungs. Panel 2, injected to the lungs, after a 10 min perfusion with Krebs solution containing 20 μg/ml of mepacrine, that does not prevent the release of RCS by arachidonic acid. Panel 3, injected to the lungs, after a 10 min perfusion with Krebs solution containing 20 μg/ml of phenylbutazone, that blocks release of RCS. Horizontal scale represents 2 min; vertical scale represents 2 cm.

phospholipase A inhibiting properties (Markus & Ball, 1969; Vargaftig & Coiron, 1970), and might act by inhibiting the enzyme once it has been activated by bradykinin and other agents; if this is its mechanism of action, it is readily understandable that it should not block RCS formation after arachidonic acid administration. Mepacrine and a few other quinolines share with phenylbutazone-like drugs and with at least one thiol derivative, penicillamine, the ability to suppress various sorts of human inflammatory conditions, and to interfere with experimental models of inflammation. Despite the variety of models no general theory of anti-inflammatory activity is available, that might integrate the effects of all antagonists. Nevertheless, when the various non-steroidal anti-inflammatory agents are considered with respect to interaction with RCS, it appears clearly that a hypothesis might be formulated, as follows: a critical point in the triggering of inflammation would be phospholipase A activation, with subsequent release of fatty acids which are precursors of RCS-like peroxides and of prostaglandins. The latter have been found in inflamed tissues (Willis, 1970). Mepacrine-like drugs would act by preventing the triggering of the process, while phenylbutazone-like drugs and thiol agents interfere in a later stage, preventing formation of the peroxide precursor of prostaglandins, or favouring a shift in the final outcome from pro-inflammatory prostaglandins to less active agents. A shift in the synthesis of $PGF_2\alpha$ to PGE_2 from arachidonic acid, or alternatively, has been reported when mono or dithiol agents are present in the incubation medium (Lands, Lee & Smith, 1970).

A necessary requirement to validate this hypothesis is that bradykinin activates endogenous phospholipase A or another acylhydrolase and that this precise enzyme should be blocked by mepacrine.

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Effects of phenoxybenzamine on transmitter release and effector response in the isolated portal vein

The output of noradrenaline from adrenergically innervated tissues is much increased at sympathetic stimulation after α-adrenoceptor blocking agents, e.g., phenoxybenzamine (PBZ) (Brown & Gillespie, 1957; Thoenen, Hürlimann & Haefely, 1964; Boullin, Costa & Brodie, 1967) and such drugs have often been used in the quantitative measurement of transmitter release (see e.g., Boullin & others, 1967; Stjärne, Hedqvist & Bygdeman, 1969; Langer & Vogt, 1971). The increased output of noradrenaline after PBZ has been ascribed to different actions of the drug, especially to blockade of the neuronal reuptake of noradrenaline and to α-adrenoceptor blockade, but there is no general consensus about the relative importance of the different mechanisms. We considered it of interest to study simultaneously the release of [^{3}H]noradrenaline (^{3}H-NA) and the degree of α-adrenoceptor blockade during nerve stimulation at graded concentrations of PBZ. The study was made *in vitro* on a thin tissue, the isolated rat portal vein, in which the conditions for transmitter diffusion are good. The release of noradrenaline was studied with the use of ^{3}H-NA (Häggendal, Johansson & others, 1970).

The basic procedure was that of Häggendal & others (1970). Three isolated portal veins from rats of the Sprague-Dawley strain were mounted in parallel and isometric recording of the mechanical activity was made from one of these vessels. After an accommodation period of 1 h in an organ bath containing a modified Krebs solution (for details see Häggendal & others, 1970) 1-^{3}H-NA was added (10^{-7}M, specific activity 2·34 Ci/mmol, Radiochemical Centre, Amersham, England). After 30 min of incubation another organ bath (volume 1·5 ml) was placed in position and the portal veins were continuously superfused at a rate of 1 ml/min with Krebs solution. The mechanical activity was recorded continuously and the superfusate was sampled throughout for measurement of total radioactivity. During the first