1-*n*-Butylimidazole: a potent and selective inhibitor of 'Thromboxane Synthetase'

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'Thromboxane Synthetase' is a membrane-bound enzyme which transforms the prostaglandin (PG) endoperoxides G_2 and H_2 to the very labile species, thromboxane (TX) A_2 (Hamberg & Samuelsson, 1974; Hamberg, Svensson & Samuelsson, 1975; Needleman, Moncada, Bunting, Vane, Hamberg & Samuelsson, 1976). In ng quantities TXA_2 causes 5-hydroxytryptamine release from platelets and considerable evidence points to a major role for TXA_2 in the irreversible aggregation of platelets caused by some stimuli. As a product of the cyclo-oxygenase pathway, TXA_2 formation is blocked by aspirin-like drugs (Vane, 1971) thus providing an explanation for the observation that aspirin prevents second phase platelet aggregation (O'Brien, 1968). Moncada, Bunting, Mullane, Thorgood, Vane, Raz & Needleman (1977) reported that TXA_2 biosynthesis was inhibited by imidazole, and (to a slightly greater degree) by 1-methyl imidazole. We now report that 1-*n*-butylimidazole is a very potent (almost 40 times more active than imidazole) and selective inhibitor of blood platelet thromboxane synthetase.

The enzymatic oxidation of arachidonic acid by lysates of horse platelets was estimated radiochemically and was used as an initial test of activity of imidazole derivatives: this technique, including details of t.l.c. systems, and methods of quantitation has been previously reported (Blackwell, Duncombe, Flower, Parsons & Vane, 1977). In some experiments the conversion of (1-^{14}C) PGH_2 to TXB_2 (the stable metabolite of TXA_2) by a 100,000 *g* fraction of horse platelet lysates was estimated in a similar manner. Platelet aggregation was measured by the turbidometric method of Born (1962).

When tested as an inhibitor of TXA_2 biosynthesis from arachidonic acid, the I_{50} concentration of 1-*n*-butylimidazole was 40 μM (imidazole 1.5 mM), and in concentrations up to 1.0 mM, did not affect the conversion of arachidonic acid into HETE or to other cyclo-oxygenase products such as PGE_2, $F_{2\alpha}$ and D_2. In fact, there was considerable stimulation of the latter products in the presence of the drug, presumably because of a surfeit of PG endoperoxides now available for other transformations.

To exclude the possibility that 1-*n*-butylimidazole was working by an indirect mechanism it was tested as an inhibitor of the conversion of PGH_2 to TXA_2. The I_{50} of 1-*n*-butylimidazole in this test was 0.42 mM—imidazole itself gave less than 5% inhibition at 1.5 mM. Kinetic experiments demonstrated that the inhibition was competitive in nature.

In a final series of experiments 1-*n*-butylimidazole was tested as an inhibitor of human platelet aggregation induced by 0.6 nmole PGH_2. Maximal inhibition of the aggregating response (about 91%) was achieved at a concentration of only 8 μM, the I_{50} being 3.5 μM (2 experiments). Increasing the concentration of the drug to 1 mM did not affect the final degree of inhibition (91%) obtained, suggesting that the endoperoxide itself has significant, albeit weak aggregating activity.

While this work was in preparation Tai & Yuan (1978) reported similar findings.

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