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Biochemical Pharmacology, Vol. 33, No. 23, pp. 3917–3918, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00

The effect of cicletanide, a diuretic, on the platelet vessel wall interaction; its involvement in the arachidonic acid cascade

(*Received* 12 *August* 1983; *accepted* 25 *June* 1984)

In previous experiments the role of the prostaglandin biochemical pathway in platelet vessel wall interaction was described [1–4] in an *in vivo* model developed in the white male Wistar rat [5, 6]. White platelet thrombus induction and registration was standardized in a branch of the mesenteric artery. Transmission and scanning electronmicroscopic data evidenced that the electrically induced lesion consists of an area of local deendothelialization approximately 150–200 μm in diameter; the surrounding endothelial cells and the smooth muscle cells do not evidence morphological changes while the lamina elastica interna remains intact.

Topical superfusion with ADP (4.10^{-4} M) results in the stimulation of platelet function which results in the adhesion of platelets onto the denuded area followed by aggregation into a thrombotic mass. These phenomena following the stimulation of platelet function depend among other possible mechanisms on the arachidonate cascade occurring in the neighbouring cells, the ratio of the pro-aggregating cyclic endoperoxides to prostacyclin seems the gearing factor [7–9].

More recently, it was evidenced that certain diuretic agents affected the prostaglandin pathway and particularly the generation of prostacyclin [10, 11]. In view of these findings, it was deemed essential to investigate the effect of cicletanide (Fig. 1) in the *in vivo* model in order to evaluate its effect on local ADP induced thrombogenesis. As topical superfusion with cicletanide (10^{-3} M) alone does not affect ADP induced thrombogenesis, the experimental protocol consists then in firstly the registration of control values for the ADP induced thrombi, then the recording of the thrombi when ADP was superfused with tranylcypromine, an inhibitor of prostacyclin synthetase and as such enhances local thrombosis. Secondly control values are again registered then followed by registration of the thrombotic parameters during superfusion with tranylcypromine and cicletanide together.

All experiments are performed with the simultaneous superfusion of ADP with arachidonic acid (10^{-4} M). Tranylcypromine and cicletanide are used at respectively 2.10^{-3} M and 10^{-3} M in order to induce by topical superfusion a sufficient gradient of concentration within the vessel wall. All animals were injected 3 hr previously with 150 mg/kg ASA (lysine salt) intravenously in order to inhibit the platelet cyclooxygenase activity, while the endothelial cell cyclooxygenase activity has recovered completely after this time interval. All solutions were made up in 5% dimethylsulfoxide and the superfusion with tranylcypromine and cicletanide is started 1 min before the ADP superfusion.

Different discriminating parameters are recorded in this investigation: the $m_{(T)}$ value which represents the maximum on the $T(t)$ curve. The $T(t)$ curve represents the sum of all electrical potentials on all LDR elements during each time interval of $30 \times 1/9 \times 10^{-2}$ sec; the $m_{(D)}$ value which represents the maximal on the $D(t)$ curve. The $D(t)$ curve represents at each $30 \times 1/9 \times 10^{-2}$ sec interval the highest potential deviation on one of the LDR elements covered by the thrombus image, the *TTV* value which is the maximal value of the $TTV(t)$ curve resulting from the integration of the $T(t)$ curve in function of time; the *TVM* value which is the value of the $TTV(t)$ curve up to the point where $m_{(T)}$ is reached.

The results are indicated in Table 1.

These findings clearly demonstrate that cicletanide antagonizes the enhancing effect of tranylcypromine on ADP induced thrombogenesis. Previous experimental data [3, 4] demonstrated that the inhibition of PGI_2 synthetase by tranylcypromine markedly enhanced ADP induced thromboformation, a phenomenon which can be explained by the platelet endothelial cell interaction involving cyclic endoperoxides. Cicletanide by itself alone does not modify ADP induced thromboformation; its antagonizing effect on the thrombosis enhancing property of tranylcypromine could be due to some activation of PGI_2 synthetase. Other authors indeed have described this effect in cultures of aortic smooth muscle cells [12] and following the i.v. admin-

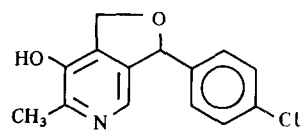

Fig. 1. Cicletanide (BN 1270): 1,3-dihydro 6-methyl 7-hydroxy 3-(4-chlorophenyl) furo (3,4-c) pyridine.

Table 1. Effect of superfusion with tranycypromine (TR, 2 mM) and cicletanide (CI, 1 mM) on thromboformation induced by ADP (400 μM; 45 sec) and arachidonic acid (100 μM)

Parameter	Control	During (TR) superfusion	Control	During (TR + CI) superfusion
$m_{(T)}$ mV	602.7 ± 51.6	987.0 ± 177.6*	680.8 ± 97.2	714.2 ± 125.1†
$m_{(D)}$ mV	184.2 ± 12.3	279.7 ± 37.0*	186.8 ± 23.0	219.8 ± 34.8†
TVM V sec	16.0 ± 1.3	55.3 ± 20.7*	16.2 ± 2.2	23.8 ± 6.3†
TTV V sec	25.1 ± 2.3	66.9 ± 21.5*	29.4 ± 5.4	33.8 ± 8.0†

The represented data are the means ± S.E.M. of 6 experimental animals, injected with 150 mg/kg of ASA intravenously 3 hr before the registration of the control values.

All solutions contain 5% DMSO (dimethylsulfoxide).

Superfusion with TR or TR + CI was started 1 min before thrombus induction and continued until complete disappearance of the thrombus. Cicletanide alone does not modify the response to ADP (5 assays).

Significance levels are obtained via the Wilcoxon matched-pairs signed-ranks test.

* $P = 0.05$.

† Not significant.

istration of cicletanide together with arachidonic acid in the rabbit [13]. This evidently does not exclude the possibility that cicletanide competes with tranylcypromine for a common receptor site.

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