PROSTAGLANDIN AND NOREPINEPHRINE METABOLISM: EFFECT OF INDOMETHACIN ON PROSTAGLANDIN SYNTHESIS AND NOREPINEPHRINE TURNOVER RATE

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Although inhibition of prostaglandin (PG) synthesis has been suggested as a mechanism of ulcerogenic action of indomethacin (IDM), the exact mode of action is by no means established. Since PG protects the gastric mucosa from ulceration by inhibiting gastric secretion (1-4) inhibition of synthesis would remove this protection mechanism (5). On the other hand, the modulatory effect of PG on sympathetic transmission has been reported (6). For example, inhibition of prostaglandin E (PGE) synthesis is accompanied by an increased release of norepinephrine (NE) in response to sympathetic nerve stimulation (7-9). NE constricts the gastric vasculature and decreases the gastric blood flow, therefore the possibility exists that increased sympathetic activity in the stomach may be involved in the pathogenesis of IDM induced gastric ulceration. The protective effect of sympathetic blocking agents against IDM ulceration (10) supports this possibility. It was, therefore, of interest to determine whether inhibition of PG synthesis by IDM could be accompanied by an increase in NE turnover rate in the stomach.

Male Wistar rats weighing 150-200 g were fasted (water ad libitum) 18 hr before experiments. IDM (20 mg/kg) was injected i.p. and the animals were sacrificed 7 hr later. After removing the spleens for enzyme assay, the stomachs were filled with about 10 ml of 1% formalin solution for 10 min. After washing with saline, the stomachs were cut along the greater curvature and the gastric ulceration was observed. The ulcer index was divided into 6 according to the method of Osumi et al. (11). PG synthetase was prepared by the method described by Vane (5) with slight modification. The spleens were excised and homogenized in 4 vol. of ice-cold modified Bucher medium using a Teflon pestle. The
homogenate was then centrifuged at 900 g for 15 min and the supernatant utilized. One ml of enzyme preparation was incubated in a glass test tube of 1 cm diameter with 10 μg arachidonic acid (0.05 ml), 5 μg hydroquinone (0.1 ml), 50 μg reduced glutathione (0.1 ml) at 37°C for 30 min with shaking. The reaction was stopped by heating the tube in boiling water for 30-60 sec. The incubation mixture was then diluted 10 times with saline and was bioassayed immediately or at least within 24 hr (stored in ice-cold water). PG-like activity was assayed in terms of PGE₂ on rat stomach strip (12), using PGE₂ as standard. The strip was superfused with Tyrode solution (13) containing a mixture of antagonists to give a final concentrations of the bases: scopolamine hydrobromide (10⁻⁷ g/ml), cyproheptadine hydrochloride (2.·10⁻⁶ g/ml) and diphenhydramine hydrochloride (2·10⁻⁷ g/ml). In order to prevent PG synthesis in the assay tissue and enhance the sensitivity, IDM (1 μg/ml) was added to the superfused solution (14). NE turnover rate in the stomach was calculated by the method described by Djahanguiri et al. (15). L-NE-7-³H (³H-NE) (8.9 mCi/mmole) hydrochloride from Radiochemical Centre, Amersham was dissolved in saline and was injected i.v. into rats at a dose of 100 μCi/kg. Two hr later, 3 rats were sacrificed, the stomach removed and the radioactivity measured as described below. Four of the remaining 7 rats were injected i.p. with DMI (20 mg/kg) and the other 3 rats were injected i.p. with saline and served as control. Seven hr later all remaining animals were sacrificed, the stomach removed and the radioactivity measured as described below. Four of the remaining 7 rats were injected i.p. with DMI (20 mg/kg) and the other 3 rats were injected i.p. with saline and served as control. Seven hr later all remaining animals were sacrificed, the stomach removed and ³H-NE was extracted as described by Anton and Sayre (16). Radioactivity in aliquots of extract was determined in 10 ml of Bray’s solution by Packard Tri-Carb liquid scintillation counter. Counting efficiency was 47%. Endogenous NE concentration in the stomach of non-treated rats was assayed spectrophotofluorometrically by the method of Anton and Sayre (16). The turnover rate was calculated from endogenous NE concentration and rate of disappearance of ³H-NE.

Seven hr after i.p. administration of IDM (20 mg/kg) ulcers were observed in the glandular portion of stomach in 17 out of 19 rats, that is, approximately 90% of the treated animals. In 1 out of the 17 rats above mentioned, only slight edema and congestion were observed, whereas erosions were quite evident in all remaining animals. The mean ulcer index was 3.8 ± 0.5 (S.E.). PGE₂ synthetic activity in spleens from the 17 rats was compared with that in spleens from 17 non-treated rats. Little PGE₂ activity could be detected in the incubation medium containing ulcerated rat spleen homogenates, that is, only 14.3 ± 7.2% (S.E.) of the control. NE turnover rate was measured both in glandular and nonglandular portions of the stomach from rats treated with IDM (20 mg/kg, i.p., 7 hr prior to sacrifice) and comparison was made with saline-treated control animals. The results in Table 1 show that there were no significant differences between IDM- and saline-treated rats in rate constant and turnover rate of NE both in glandular and nonglandular portions of the stomach.

The present results suggest that IDM, at a dose which produces considerable gastric ulceration, does not facilitate the release of NE from sympathetic nerve terminals in the stomach. There are two possible explanations for this: 1) In certain autonomic effector systems the modulatory role of PG may well involve the balance between the depressant effect of PGE and the facilitatory effect of PGF (17). Since IDM inhibits synthesis of PGE₂,
TABLE 1. Turnover rate of norepinephrine in stomach from rats treated with indomethacin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>NE level (ng/g ± S.E.)</th>
<th>Rate constant (k/hr⁻¹ ± S.E.)</th>
<th>Turnover rate (ng/g/hr ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glandular stomach</td>
<td>Control</td>
<td>368±10 (15)</td>
<td>0.064±0.013 (3)</td>
<td>23.67±5.05 (3)</td>
</tr>
<tr>
<td></td>
<td>Indomethacin  (20 mg/kg)</td>
<td>369±0.027 (4)</td>
<td></td>
<td>25.66±10.13 (4)</td>
</tr>
<tr>
<td>Nonglandular stomach</td>
<td>Control</td>
<td>371±20 (15)</td>
<td>0.062±0.007 (3)</td>
<td>23.12±2.95 (3)</td>
</tr>
<tr>
<td></td>
<td>Indomethacin  (20 mg/kg)</td>
<td>369±0.012 (4)</td>
<td></td>
<td>22.25±4.47 (4)</td>
</tr>
</tbody>
</table>

Values given are the mean±S.E. The number of animals is given in parentheses.

as well as that of PG₂, the balance between the effects of two PG groups on sympathetic activity is likely to be unchanged. 2) IDM is an inhibitor of catecholamine (CA) uptake in synaptosomes from the central nervous system (CNS) (18). If such is the case in sympathetic nerve terminals in the stomach, the inhibition of NE uptake results in an increase of NE concentration at receptor site and thus, by a feedback mechanism, decreases the rate of disappearance of ³H-NE from presynaptic neurons.

Bhargava et al. (19) reported that the ulcerogenic activity of IDM was due to a release of CA from the adrenal medulla as the result of activation of the CNS. Therefore, the increased release of NE from nerve terminals in any organ other than the stomach may contribute to the pathogenesis of IDM-induced gastric ulceration. Siggins (20) reported that PG₂, E₂ and F₂, antagonize the vasoconstriction effect of epinephrine and NE in frog retrolingual membrane. Therefore, the possibility that sensitivity of the gastric vascular system to NE is increased by decreased PG synthesis, thereby reducing gastric blood flow should be considered.

In summary, IDM, at a dose which produces considerable gastric ulceration, did not increase NE turnover rate in rat stomach.

REFERENCES