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The Effect of Dinoprost on Transport of Water and Imipramine Through Rat Small Intestinal Membranes

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Abstract—The relation between transmucosal fluid movement and its effect on absorption and exsorption of imipramine was studied with the in-situ single-pass perfusion technique in rats. Dinoprost (prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$) caused a dose-related inhibition of both absorption and secretion of water across the intestinal membrane. When $PGF_{2\alpha}$ was infused at a rate of 5 μmol kg^{-1} h^{-1}, the absorption rate of water decreased from 51·7 to 21·5 mL h^{-1} and the secretion rate decreased from 48·9 to 26·8 mL h^{-1}. Net water flux changed from net water absorption (0·9 mL h^{-1}) to net water secretion (5·33 mL h^{-1}) by infusion of $PGF_{2\alpha}$. However, absorption and exsorption of imipramine were little affected by infusion of $PGF_{2\alpha}$. The absorption rates of imipramine were 3·03 and 2·36 mg h^{-1} in the absence and presence of $PGF_{2\alpha}$, respectively. Furthermore, the average amounts of imipramine exsorbed into the intestinal lumen in 2 h were 7·82 and 8·10% in the absence and presence of $PGF_{2\alpha}$, respectively. Infusion of $PGF_{2\alpha}$ also enhanced motility of the small intestine compared with the control. From these results, it appears that $PGF_{2\alpha}$ has no effect on the absorption and exsorption of imipramine across the intestinal membrane although it is reasonable to use $PGF_{2\alpha}$ in the case of patients with overdoses of drugs which decrease gastrointestinal motility.

In acute drug poisoning, the drug should be removed as soon as possible before it is absorbed from the gastrointestinal tract. In general, procedures such as gastric lavage or induction of emesis with syrup of ipecac are performed, and subsequently administration of activated charcoal with cathartics or agents which promote intestinal motility are used as methods of gastrointestinal decontamination. However, some drugs such as anticholinergic drugs, tricyclic antidepressants, antihistamines and opiates decrease bowel motility (Litovitz et al 1987). Accordingly, in the case of overdoses of these drugs, it is difficult to discharge drug-charcoal complexes from the lumen of the intestine since these complexes remain in the lumen for a long time. Prolonged transit times may result in desorption of poisoned drugs from activated charcoal. Therefore, it is important to use a means that promotes rapid removal of the toxic drug.

Dinoprost ($PGF_{2\alpha}$) is often used in patients with markedly depressed or absent bowel sounds and in patients with overdoses of drugs which decrease gastrointestinal motility, as it stimulates and enhances intestinal motility (Jaffe 1979). In addition, it reverses net absorption of water and electrolytes into the small intestinal mucosa (Pierce et al 1971). Such a change of fluid transport may affect absorption or secretion of drugs via the gastrointestinal membrane. However, there is little information to show how great the effects of $PGF_{2\alpha}$ are on intestinal absorption and/or exsorption of drugs. Therefore, it is interesting to study whether $PGF_{2\alpha}$ can affect transport of drugs across the membrane in poison cases where ingested drugs may alter gut motility.

There are several reports on interactions between water and drugs which demonstrate a solvent drag effect on drug (Ochsenfahrt & Winne 1974a, b; Kitazawa et al 1975; Karino et al 1982). For example, Ochsenfahrt & Winne (1974a, b) have demonstrated that the absorption of acidic drugs, such as benzoic acid and salicylic acid, and basic drugs, such as amidopyrine, was increased when net water absorption was increased. Kitazawa et al (1975) have also shown that absorption of sulphanilamide, sulphafurazole and metoclopramide was increased with increasing transmucosal fluid movement from the lumen to the blood and decreased when the movement of water was directed from the blood to the lumen in the rat small intestine. Furthermore, they reported that exsorption of sulphanilamide was increased with an increase in tonicity of the perfusate, namely with an increase in transmucosal fluid movement from the blood to the lumen, in the rat small intestine (Kitazawa et al 1977).

The aim of the present work was to investigate the effect of $PGF_{2\alpha}$ on the absorption and exsorption of a drug in overdoses. A tricyclic antidepressant, imipramine, was used as the representative drug. This drug is frequently encountered and causes a decrease in peristalsis in acute drug poisoning.

Materials and Methods

Materials

Imipramine hydrochloride was supplied by Ciba-Geigy (Japan) Co., Takarazuka, Japan. $PGF_{2\alpha}$ for injection (Prostarmon·F) was a product of Ono Pharmaceutical Co., Osaka, Japan. ^3H_2O was purchased from Amersham, Tokyo, Japan. Scintillation fluid (Instagel) was from United Technologies (Packard, Illinois, USA). All other chemicals used in this study were of analytical grade.

Absorption and exsorption study

Intestinal absorption and exsorption experiments were performed by an in-situ single pass perfusion technique (Arimori & Nakano 1985). Wistar strain male rats, 200–300 g, were anaesthetized by i.p. injection of ethyl carbamate (1·2 g kg^{-1}). The small intestine was exposed by midline abdominal incision. The upper duodenum and the ileocaecal junction

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Table 3. C^3-CH_3 and C^5- ^{13}C NMR resonance for some substituted piperidines.

Compound	C^3-CH_3 (ppm)	C^5 (ppm)
3a	11·69	31·77
3b	14·68	25·41
5a	12·65	39·70
5b	14·83	31·89
6a	12·36	40·59
6b	15·89	31·40
7a	12·45	40·25
7b	16·38	33·44

* In $CDCl_3$, TMS internal standard. *4a,b* were omitted because they lacked $CDCl_3$ solubility. The resonance positions of interest differed little between the isomers in other solvents (DMSO-d_6; CD_3OD).

diastereoisomer *7b* was ineffective at an i.v. dosage of 2·5 mg kg^{-1} (failure of a compound to elicit signs of an antinociceptive response at this dose level corresponds to a high ED50 value) (Schellekens, private communication). This result contrasts with observations made on the prodines, where greater activity is seen in the β-form (Casy 1973). However, in tests on antipodal forms of the α-diastereoisomer *7a*, activity was found to reside in the 3*R*, 4*S* isomer in agreement with results noted for antipodes of α-prodine (the 3*R*, 4*S* isomer was about 25 times as active as the 3*S*, 4*R* form in the mouse hot-plate test) (Larson & Portoghese 1973).

In the mouse tail-flick test, 3*R*, 4*S*-*7a* displayed twice the activity of (±)-*7a*, and its antipode was inactive. Acetylation of (±)-*7a* lowered its potency in the rat test to 2·5 mg kg^{-1}— in the prodines an acyl group is an essential requirement for activity. The difference in activity between the *t*-alcohol *7a* and the ester *8a* is small and may be the result of pharmacokinetic factors.

Under the in-vitro conditions of the mouse vas deferens test, (±)-*7a* proved several times less effective than morphine suggesting that its superior potency in the rat tail-withdrawal procedure might be due to its greater ease of penetration of the central nervous system. Its action in the mouse vas deferens test resembled that of a μ-ligand since its effects were not seen in tissue pretreated with βFNA (the amide of fumaric acid mono-methyl ester and β-naltrexamine which selectively blocks μ-sites) but were unchanged in the presence of the selective δ-antagonist ICI-174864 (*N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH). The (±)-α preparation *7a* displaced tritiated etorphine (a universal opioid ligand) from rat brain membranes, but competed less well than morphine, in agreement with results of the mouse vas deferens experiment. Its K_i value vs the selective μ-ligand DAGO (D-Ala^2, $MePhe^4$, Gly-ol^5]enkephalin) was a tenth of that against etorphine, as further evidence of its μ-affinity.

The affinity of (±)-*7a* for phencyclidine receptors was very low, since it failed to displace the selective ligand [^{3}H]TCP (1-[1-(2-thienyl)cyclohexyl]piperidine) from rat brain membranes at a concentration of 1000 nM (cf phencyclidine, $K_i = 60$ nM) (Sircar & Zukin 1985).

It is concluded that the hybrid phencyclidine/4-aryl piperidines, *7*, associate with opioid (probably of the μ-subtype) rather than phencyclidine receptors, but with a binding mode that differs from that of pethidine reversed esters. It is possible that derivatives, *7*, may relate more closely to opioids in which 4-phenyl-4-piperidinol is linked through nitrogen to a CH_2 CH (H or CH_3) N (COEt) Ph chain (activity was also reduced on *O*-acylation) (Carabateas et al 1963; Fancher et al 1964).

Table 4. Biological activity data for phencyclidine analogues.

Compound	TWR[a] ED50 (mg kg^{-1} i.v.)	TFM[b] ED50 (mg kg^{-1} s.c.)	Binding[c] EC50 (nM)	MVD[d] EC50 (mM)
(±)-*7a* (α)	1·0	15·3 (5·7–41·1)	680	1·42[e]
3*R*, 4*S* (+)-*7a*	1·25	8·7 (4·1–18·3)	—	—
3*S*, 4*R* (−)-*7a*	>2·5	essentially inactive	—	—
(±)-*7b* (β)	>2·5	—	—	—
(±)-*8a*	2·5	—	—	—
Morphine	3·15	5·8	23·6	0·395

[a]Rat tail-withdrawal test (Janssen et al 1963). [b]Mouse tail-flick, (Dewey et al 1970). [c]Using 0·5 nM [^{3}H]etorphine in cerebral membranes from rat brain suspended in 50 mM Tris HCl buffer (pH 7·4) containing 150 nM NaCl (Woods et al 1988). [d]Mouse vas deferens test (Woods et al 1988). [e]Action blocked by naltrexone.

Preparative Work

^{13}C NMR spectra were recorded at 67·8 MHz using a Jeol GX270 MHz NMR spectrometer. The number of protons associated with each carbon atom was established from DEPT experiments. ^1H NMR spectra were recorded on a Jeol GX270 spectrometer. Unless stated otherwise, TMS was employed as internal standard, and $CDCl_3$ as solvent. Routine ^{13}C and ^1H NMR data, consistent with structure in all cases, are not quoted and may be obtained from the authors on request. Abbreviations for data quoted are: d, doublet; t, triplet; q, quartet; m, multiplet, plus combinations of dt, doublet of triplets.

Infrared spectra, recorded for liquids as films and for solids as KBr discs, were obtained using a Unicam SP1020 spectrometer.

Optical rotation readings were recorded on an Optical Activity Ltd AA-10 Polarimeter at the sodium D line (589 nm).

Elemental analyses were performed by Butterworth Laboratories Ltd, Middlesex and the Chemistry Department, University of Bath. Melting points are uncorrected.

Starting materials

α- and β-Prodine were obtained starting from 1,3-dimethylpiperidone (Howton 1945) by the method of Ziering & Lee (1947). α-Prodine was resolved into its (+) and (−) antipodes by crystallization of (+)- and (−)-tartrate salts, respectively, by the method described by Larson & Portoghese (1973).

(+)-*3a*, (+)-Tartrate salt: mp 163–164°C, $[\alpha]_D^{25}$ +13·5°C (c = 1, H_2O).

[Lit. (Larson & Portoghese 1973) mp 162–163°C, $[\alpha]_D^{25}$ +13·5° C (c = 1, H_2O)].

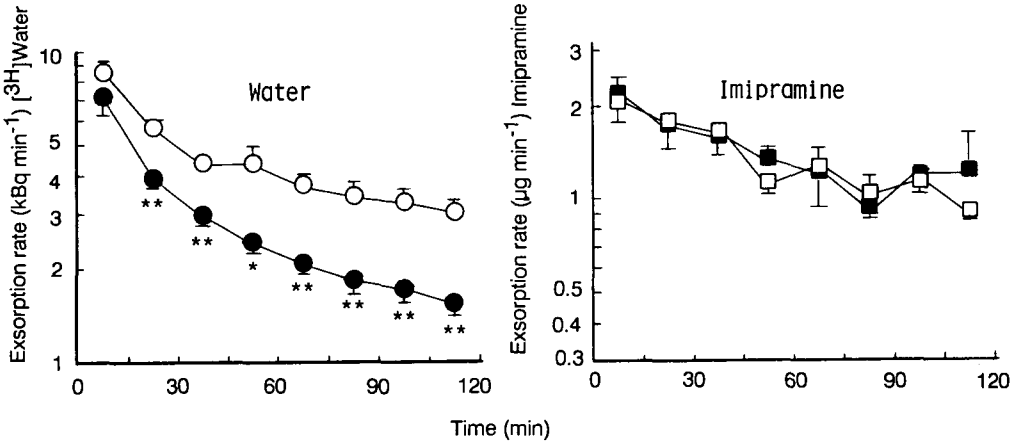

FIG. 2. Effect of presence (●, ■) and absence (○, □) of $PGF_{2\alpha}$ (10 μmol kg^{-1} h^{-1}) on the exsorption rate of water and imipramine from the blood into the intestinal lumen after i.v. administration of tritiated water (1·85 MBq) and imipramine (10 mg kg^{-1}) to rats. Each point represents the mean ± s.e.m. of 4 rats. * $P < 0.05$, ** $P < 0.01$.

Table 2. Effect of $PGF_{2\alpha}$ (10 μmol h^{-1} kg^{-1}, i.v. infusion) on charcoal transit in 1 h in rat small intestine.

	Body weight (g)	Length of small intestine (L) (cm)	Transit distance (T) (cm)	Ratio T/L %
Control	203 ± 6·67	80·3 ± 4·84	19·0 ± 3·79	23·3 ± 3·40
Treatment with $PGF_{2\alpha}$	198 ± 6·01	85·7 ± 3·48	56·3 ± 10·9*	64·7 ± 12·0*

Each value represents the mean ± s.e.m. of 3 rats. * $P < 0.05$.

significantly decreased absorption of water, while imipramine was not significantly affected.

Fig. 2 shows the exsorption patterns of water and imipramine from the blood into the perfusate across the small intestinal mucosa following i.v. administration of impramine at a dose of 10 mg kg^{-1} to rats in the presence or absence of $PGF_{2\alpha}$ infusion. Infusion of $PGF_{2\alpha}$ significantly decreased exsorption of water. The average amounts of tritiated water exsorbed into the intestinal lumen in 2 h were 29·6% of the dose in the control and 19·4% during the treatment with $PGF_{2\alpha}$. The average amounts of imipramine exsorbed into the intestinal lumen in 2 h were 7·82% of the dose in the control and 8·10% during the treatment with $PGF_{2\alpha}$.

Effect of $PGF_{2\alpha}$ on intestinal motility

The effect of $PGF_{2\alpha}$ on intestinal motility in rats was examined by using activated charcoal as a transit marker. The results are shown in Table 2. It was observed that $PGF_{2\alpha}$ significantly enhanced the motility of the small intestine. The extent of charcoal transit during the treatment with $PGF_{2\alpha}$ was 64·7% of the total length of the small intestine and significantly greater than that in the control (23·3%).

Discussion

It is important to use a drug which enhances gastrointestinal motility to hasten removal of the activated charcoal-drug complex from the bowel in drug overdoses. $PGF_{2\alpha}$ may enhance elimination of the toxic drug from the body since this drug causes an increase in the gut motility (Table 2). Our results also showed that infusion of $PGF_{2\alpha}$ inhibited not only absorption but also secretion of water from the blood to the lumen and reversed net water absorption to secretion in a dose-dependent manner in rats. It has been shown that several lipid soluble compounds affect absorption and/or exsorption with change in the transmucosal fluid movement by the solvent drag (Ochsenfahrt & Winne 1974a, b; Kitazawa et al 1975; Karino et al 1982). Infusion of $PGF_{2\alpha}$, however, caused little effect on both absorption and exsorption of imipramine in the present study. The reason for the lack of the effect of $PGF_{2\alpha}$ on the transport of imipramine remains to be elucidated.

In general, the absorption rate of drugs depends on the luminal concentration, resistances of the mucosal unstirred water layer and the intestinal epithelium and on the blood flow. Of these factors, the concentration gradient of the drug between the blood and the lumen is always kept large in the in-situ single-pass perfusion experiments. Furthermore, the blood flow appears to be little affected by $PGF_{2\alpha}$ since it has little vascular effect although alprostadil (PGE_1) and PGA are potent vasodilators which increase the blood flow by relaxation of the vascular smooth muscle (Jaffe 1979). The discrepancy between our results and previous studies may be due to methodological differences causing the solvent drag. Our experimental conditions which cause the solvent drag are different to those of Ochsenfahrt & Winne (1974a, b), Kitazawa et al (1975) and Karino et al (1982). The driving force of intestinal water movement in our study was induced by the pharmacological effect of $PGF_{2\alpha}$ since the osmolality and ionic composition of the perfusate were about the same as those of plasma, while in previous studies, water move-

ment was caused by placing hypo-, iso-, or hypertonic solutions in the lumen.

The intestinal absorption of lipid soluble drugs is influenced by the unstirred water layer. The flux of drugs across the unstirred water layer is inversely proportional to the thickness of this layer. Huang (1990) has reported that the intestinal clearance of the two lipophilic compounds, quinidine and thiopentone was decreased by addition of pectin which increases the thickness of the unstirred water layer to the intestinal perfusate. Conversely, reduction of the thickness of the unstirred water layer would enhance flux of drugs across the gastrointestinal membrane. Csaky (1984) has suggested that drugs such as $PGF_{2\alpha}$ which may increase the intestinal and villous motility will decrease the thickness of the unstirred water layer which favours a more rapid drug absorption. In contrast, Moody & Zalewsky (1981) have suggested that prostaglandins, which increase the thickness of the unstirred water layer, serve as an important protective mechanism to remove noxious substances from the mucosal surface.

It is known that tricyclic antidepressants undergo enterohepatic circulation and appreciable amounts are secreted into bile (Matthew 1972). We have also shown that about 8% of administered imipramine was transferred into the intestinal lumen in 2 h. In spite of such an appreciable transport into the lumen, it is said that oral administration of activated charcoal had little effect on the serum half-life or systemic clearance of imipramine, presumably because of its large volume of distribution (Goldberg et al 1985). Thus, it seems that the gastrointestinal dialysis by charcoal may not enhance clearance of imipramine which has already been absorbed into the systemic circulation from the gastrointestinal tract, but can inhibit absorption of impramine which has remained in the lumen because of excellent adsorbability of imipramine onto charcoal (Rauws & Noordwijk 1972). Accordingly, it is reasonable to use $PGF_{2\alpha}$ to stimulate intestinal motility and hasten excretion of an activated charcoal-toxin complex from the bowel in order to prevent desorption of toxins from activated charcoal.

In conclusion, use of $PGF_{2\alpha}$ may be of greater therapeutic value for treating overdose of drugs with anticholinergic effects, but it appears that $PGF_{2\alpha}$ would not inhibit absorption of imipramine with a decrease in absorption of water nor would it enhance exsorption of the drug with an increase in net water secretion.

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