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Protective effect of salicylate against 2,4-dinitrophenol-induced protein thiol loss in the small intestine of rats

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Abstract—The effect of the content of protein thiols and non-protein thiols in the tissue of the rat small intestinal loop on the absorption of cefmetazole has been investigated. 2,4-Dinitrophenol (DNP), at a concentration of 500 μM, caused rapid protein thiol loss, followed by non-protein thiol loss, along with a decrease of cefmetazole absorption by the intestine. The coadministration of 75 mM salicylate with DNP inhibited the effect of DNP on protein thiols and cefmetazole absorption but not on non-protein thiol loss by DNP.

It has been reported that salicylate as well as 2,4-dinitrophenol (DNP) acts as an uncoupler in uncoupling oxidative phosphorylation in mitochondria (Brody 1956). It has also been reported that NAD(P)H oxidation initiated by treatment with uncouplers induced the loss of thiol groups such as glutathione (Powis et al 1984).

We have recently shown that diethyl maleate at relatively low concentrations (up to 5 mM) increased the intestinal absorption of hydrophilic drugs such as cefmetazole with non-protein thiol loss (Nishihata et al 1986). However, we have also reported that diethyl maleate at relatively high concentration (10 to 15 mM) induced both protein thiol loss and a decrease in the intestinal absorption of hydrophilic drugs, in spite of significant non-protein thiol loss (Nishihata et al 1987).

Salicylate enhances intestinal drug absorption strongly in the colon and rectum but weakly in the small intestine (Nishihata et al 1980, 1983, 1984a, 1986; Suzuka et al 1985, 1987). Thus, it is of interest to investigate whether a strong uncoupler, such as DNP, changes the intestinal mucosal membrane permeability to hydrophilic drugs.

In the present study, we investigated the small intestinal absorption of cefmetazole, a model hydrophilic drug, in the presence of DNP and/or salicylate. We also investigated the effect of both uncouplers on thiols in the intestinal tissue.

Materials and methods

2,4-Dinitrophenol (DNP) and sodium salicylate were obtained from Sigma Inc. (St. Louis, USA) and Nakarai Chemical (Kyoto, Japan), respectively. Sodium cefmetazole was supplied by Sankyo Co. (Tokyo, Japan). Other reagents used were of analytical grade.

Male wistar rats, 200–250 g, were fasted for 16 h before experiments. During the experiments rats were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.p.) and were kept on a warm surface at 38 °C.

An absorption study of cefmetazole was performed using the ligated intestinal loop (Nishihata et al 1986). Briefly, an approximately 10 cm intestinal segment was ligated at the proximal end 20 cm from the bile duct after abdominal incision. After administration of 200 μL of a drug solution (15 mg of sodium cefmetazole mL^{-1}) into the ligated intestinal lumen of the loop, blood was collected from the jugular vein at designated times and centrifuged to obtain plasma which was subsequently assayed. The drug solution administered into the lumen was prepared with 0·05 M sodium phosphate buffer (pH 7·0) and was adjusted to $\mu = 0{\cdot}75$ ionic strength with sodium chloride. It has been reported that a disruption of the mucin layer of rat intestinal lumen occurs on administration of a high ionic strength solution prepared with sodium chloride or by chelating agents (Kowalewski et al 1969; Miyake et al 1985; Suzuka et al 1986). In fact, the disruption of the mucin layer caused a slight increase in the absorption of cefmetazole (not significant), probably by reducing the mucin layer thereby facilitating diffusion (Miyake et al 1985). The disruption of the mucin layer resulted in a standard deviation for cefmetazole absorption compared with the study in which the mucin layer was not distrupted (Miyake et al 1985; Suzuka et al 1986). In the present study, a high ionic strength sodium chloride solution was used, therefore, to obtain reproducible data. Assay of cefmetazole was performed by a high performance liquid chromatography method (Nishihata et al 1984b).

In a separate study, a ligated intestinal segment was removed at a designated time after administration of the solution containing 500 μM DNP and/or 75 mM salicylate and was homogenized with 0·1 M sodium phosphate buffer (pH 7·0) after rinsing with saline. Assay of non-protein thiol and protein thiol concentrations was performed by the method of Di Monte et al (1984).

Results

Administration of 500 μM DNP caused a rapid protein thiol loss followed by small loss of non-protein thiol from rat small intestinal tissue. However, the concentration of both thiols returned to normal at 5 h after administration of the DNP (Fig. 1A, B). Coadministration of 75 mM salicylate with 500 μM DNP inhibited the rapid protein thiol loss at the early stage, but did not affect the changes of non-protein thiol. Salicylate (75 mM) alone did not affect either type of thiols in the intestinal tissue.

Coadministration of 500 μM DNP with cefmetazole, or administration of DNP 1 h before the administration of cefmetazole, decreases the plasma cefmetazole concentration significantly (Fig. 1C). Since administration of cefmetazole 5 h after DNP administration showed similar plasma cefmetazole concentration profiles to those obtained after administration of cefmetazole alone, protein thiol loss caused by DNP seems related to the decrease in intestinal cefmetazole absorption.

Coadministration of 75 mM salicylate with 500 μM DNP in the solution of cefmetazole inhibited the effect of DNP in decreasing plasma cefmetazole concentration (Fig. 1D). These results may also support the concept that protein thiol loss from intestinal tissue inhibited cefmetazole absorption.

Discussion

It has been reported that non-protein thiol loss from intestinal tissue by agents such as diethyl maleate and ethanol caused an increase in intestinal absorption of hydrophilic compounds such

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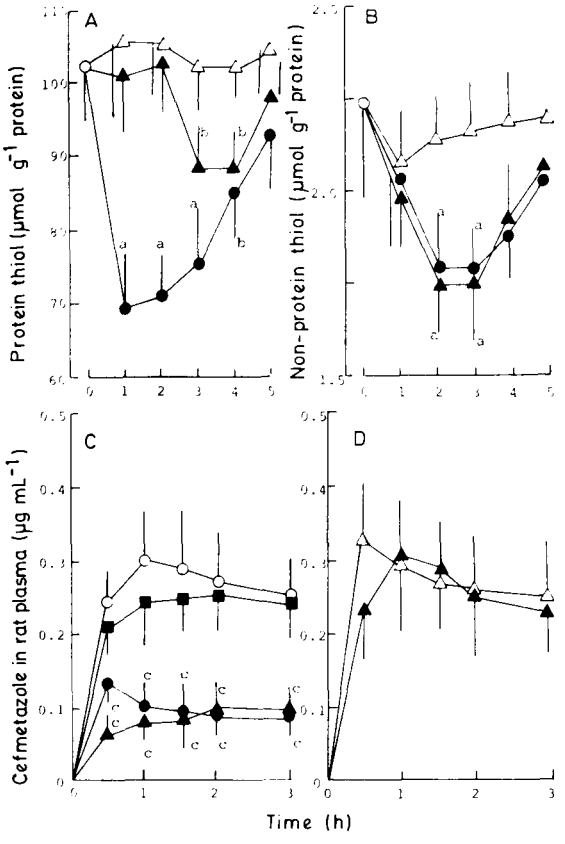

FIG. 1. (A and B) The effects of DNP and/or salicylate on protein thiol (A) and on non-protein thiol (B) concentrations in small intestinal tissue of the rat are shown as a function of time after administration into the ligated loop. Symbols area as follows: ●, administration of 500 μM DNP; △, administration of 75 mM salicylate; ▲, administration of 500 μM DNP and 75 mM salicylate. (C and D) The effects of DNP and/or salicylate on plasma cefmetazole concentration are shown as a function of time after administration of cefmetazole at a dose of 3 mg in a rat. Symbols are as follows; in (C), ○, cefmetazole alone; ●, cefmetazole given simultaneously with 500 μM DNP; ▲, cefmetazole administered 1 h after DNP administration; ■, cefmetazole administered 5 h after DNP administration: in (D), △, cefmetazole administered simultaneously with 75 mM salicylate; ▲, simultaneously with DNP and salicylate. Each value represents the mean ± s.d. (n = 5 to 6). a, $P < 0.01$ versus before administration, b, $P < 0.05$ versus before administration, and c, $P < 0.01$ versus after administration of cefmetazole alone.

as cefmetazole (Nishihata et al 1986). It has also been reported that protein thiol loss in the presence of diethyl maleate, even when non-protein thiol loss was observed, resulted in less transport of hydrophilic compounds through the intestine (Nishihata et al 1987). It might be suggested that disulphide bridge formation along with protein thiol loss reduced the diffusivity of the solute in the tissue.

Uncouplers, such as DNP, are known to change the redox status following NAD(P)H depletion in mitochondria. The loss of the thiol group after treatment with 500 μM DNP in the present study may be due to the depletion of NAD(P)H which inhibited the oxidation of the thiol group (Powis et al 1984). However, 75 mM salicylate did not affect the levels of either protein or non-protein thiol. Furthermore, it was observed in the present study that salicylate inhibited protein thiol loss induced by DNP at an early stage, despite having no influence on non-protein thiol loss. Thus, it appears that rapid protein loss caused by DNP shortly after its administration occurs by a mechanism which is independent of oxidation, when the non-protein thiol loss and protein thiol loss by DNP observed in the presence of salicylate is due to oxidation, which is NAD(P)H-dependent. It has been reported that NAD(P)H loss and non-protein thiol loss increased the concentration of cytosolic calcium ions in hepatocytes by depleting the intracellular calcium pool (Blackmore et al 1978). It has also been reported that rapid protein thiol loss from intestinal tissue caused by diethyl maleate was restored by the presence of calmodulin antagonists such as phenothiazine, which did not inhibit non-protein thiol loss (Nishihata et al 1987). Thus, it is suggested that the effect of salicylate in inhibiting protein thiol loss by DNP is due to the effect of salicylate in inhibiting calmodulin activity, probably by an effect of salicylate in interacting with calcium ion (Kunz et al 1971), which induces a decrease in free calcium ion concentration in cytosol.

The absorption of cefmetazole, a hydrophilic compound, from the intestine was decreased along with protein thiol loss by treatment with DNP, the recovery of protein thiol also resulted in the recovery of cefmetazole absorption. Salicylate inhibited the effect of DNP in decreasing cefmetazole absorption, and this effect of salicylate seems to be due to its effect in inhibiting protein thiol loss. Although salicylate is known to enhance the intestinal absorption of hydrophilic drugs as mentioned earlier, 75 mM salicylate appears to be too low a concentration to enhance cefmetazole absorption in the rat small intestine.

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References

Blackmore, P. F., Brunely, F. T., Murkes, J. C., Extone, J. H. (1978) α-Adrenergic activation of phosphorylase in liver cells involves mobilization of intracellular calcium without influx of extracellular calcium. J. Biol. Chem. 253: 343–350

Brody, T. M. (1956) Action of sodium salicylate and related compounds on tissue metabolism in vitro. J. Pharmacol. Exp. Ther. 117: 39–51

Di Monte, D., Bellom, G., Thor, H., Nicotera, P., Orrenius, S. (1984) Menadione-induced toxicity is associated with protein thiol oxidation and alteration in intracellular calcium homeostasis. Arch. Biochem. Biophys. 235: 343–352

Kowalewski, K., Chmura, G., Dent, C., Schier, J. (1969) Experimental deficiency of gastric "mucus barrier". Its effect on gastric secretion and on the formation of acute gastric ulcer in rats infused with histamine for 24 hours. Am. J. Dig. Dis. 14: 788–796

Kunz, H., Rehboch, G., Vogt, W. (1971) Absorption of salicylate and its isomers of the rat jejunum. Naunyn-Schmiedeberg's Arch. Pharmacol. 273: 331–337

Miyake, M., Nishihata, T., Nagano, A., Kyobashi, Y., Kamada, A. (1985) Rectal absorption of [Asu[1,7]]-eel calcitonin in rats. Chem. Pharm. Bull. 33: 740–745

Nishihata, T., Rytting, J. H., Higuchi, T. (1980) Enhancement of rectal absorption of drug by adjuvants. J. Pharm. Sci. 69: 744–745

Nishihata, T., Takahagi, H., Higuchi, T. (1983) Enhanced small intestinal absorption of cefmetazole and cefoxitin in rats in the presence of non-surfactant adjuvants. J. Pharm. Pharmacol. 35: 124–125

Nishihata, T., Rytting, J. H., Higuchi, T., Caldwell, L. J., Selk, S. J. (1984a) Enhancement of rectal absorption of water-soluble antibiotics in dogs. Int. J. Pharm. 21: 239–248

Nishihata, T., Takahagi, H., Yamamoto, M., Tomida, H., Rytting, J. H., Higuchi, T. (1984b) Enhanced rectal absorption of cefmetazole and cefoxitin in the presence of epinephrine metabolites in rats and a high-performance liquid chromatographic assay of cephamycin antibiotics. J. Pharm. Sci. 73: 109–112

Nishihata, T., Nghiem, B. T., Yoshitomi, H., Lee, C-S., Dillsaver, M., Higuchi, T., Choh, R., Suzuka, T., Furuya, A., Kamada, A. (1986) Changes in intestinal mucosal permeability caused by nonprotein thiol loss in rats. Pharm. Res. 3: 345–351

Nishihata, T., Suzuka, T., Furuya, A., Yamazaki, M., Kamada, A. (1987) Influence of diethyl maleate-induced loss of thiols on cefmetazole uptake into isolated epithelial cells and on cefmetazole absorption from ileal loop of rats. Chem. Pharm. Bull. 35: 2914–2922

Powis, G., Svingen, B. A., Dahlin, D. C., Nelson, S. D. (1984) Enzymatic and nonenzymatic reduction of *N*-acetyl-*p*-benzoquinone imine and some properties of the *N*-acetyl-*p*-benzoquinone imine radical. Biochem. Pharmacol. 33: 2367–2376

Suzuka, T., Nishihata, T., Yamazaki, M., Kamada, A. (1985) Effect of salicylate and disodium ethylenediaminetetraacetic acid on the rat intestinal absorption of cefmetazole. Chem. Pharm. Bull. 33: 4600–4605

Suzuka, T., Furuya, A., Kamada, A., Nishihata, T. (1986) Effect of phenothiazine and W-7 on rat colonic absorption of cefmetazole. J. Pharmacobiodyn. 9: 460–465

Suzuka, T., Furuya, A., Kamada, A., Yamazaki, M., Nishihata, T. (1987) Effect of diethyl maleate and sodium salicylate on cefmetazole transport in the in vitro rat everted intestinal sac: influence of Ca^{2+} and vanadate in the medium. Chem. Pharm. Bull. 35: 1619–1623

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Brain monoamines during carrageenan-induced acute paw inflammation in rats

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Abstract—Paw inflammation was induced in rats by sub-plantar administration of carrageenan. Significant inflammatory oedema was observed 1 h later and the peak effect was noted between 3–4 h. The oedema was markedly reduced after 12–24 h. Steady state levels of whole brain and hypothalamic monoamines were estimated spectrofluorometrically during the course of the carrageenan-induced paw inflammation. In addition, the rate of accumulation of the brain 5-hydroxytryptamine (5-HT) and noradrenaline (NA) was assessed in clorgyline-pretreated rats during the inflammation. The whole brain and hypothalamic concentrations of 5-HT and NA were augmented during the early phase of the inflammation, but fell below control values when peak inflammation was achieved. Thereafter, the monoamine levels tended to normalize by 24 h when the inflammation had virtually subsided. On the contrary, whole brain and hypothalamic dopamine levels remained largely unaffected. The rate of accumulation of brain 5-HT and NA were enhanced during carrageenan inflammation, indicating that the turnover of these monoamines is augmented during the inflammatory process. The results suggest that acute peripheral inflammation may significantly affect central 5-HT and noradrenergic activity in rats.

The cascade of events which initiate, maintain and terminate peripheral inflammation is now fairly well elucidated (Bonta 1978). However, little is known about the role of the central nervous system (CNS), if any, in the putative modulation of peripheral inflammation (Bonta 1978). Schizophrenics have an unusually low incidence of rheumatoid arthritis and have shown attenuated inflammatory response to injury or infection (Horrobin 1977). Acute inflammatory oedema is inhibited by general anaesthetics (Griswold et al 1982; Bhattacharya et al 1987) and narcotic analgesics (Bonta 1978). In recent reports from this laboratory, carrageenan-induced acute paw oedema in rats was shown to be attenuated following the central administration of NA (Bhattacharya & Das 1986), 5-HT (Bhattacharya & Das 1985a), histamine (Bhattacharya & Das 1985b), prostaglandin (PG) $F_{2\alpha}$ (Bhattacharya & Das 1984a), GABA and glycine (Bhattacharya & Sarkar 1986). On the contrary, the central administration of acetylcholine (Das & Bhattacharya 1985), PGE_2 (Bhattacharya & Das 1984a) and glutamic acid (Bhattacharya & Sarkar 1986), augmented the inflammatory oedema. These findings indicate that central neurotransmitter systems can modulate acute peripheral inflammation.

It is known that acute inflammation is often self-limiting and it has been postulated that this is a consequence of automodulatory processes initiated by the inflammatory process (Bonta 1978). Since several neurotransmitter systems do appear to exert an anti-inflammatory effect, it is possible that at least part of this proposed automodulatory process results from activation of these central neuroregulators. However, no attempt appears to have been made to investigate the effect of acute peripheral inflammation on central neurotransmitter activity, apart from a report which shows that rat brain PGE_2 and $PGF_{2\alpha}$ levels are augmented during carrageenan-induced paw inflammation (Bhattacharya & Das 1984b). We now report the effect of carrageenan-induced inflammation on rat brain monoaminergic activity.

Materials and methods

The studies were conducted on male Wistar strain albino rats (120–180 g), housed in colony cages at an ambient temperature of 25±2 °C and 45–55% relative humidity, with a 12 h light-dark cycle. The rats had free access to standard pellet chow and water. Experiments were conducted at this ambient temperature between 0900 and 1400 h.

Inflammation of the paw was induced by carrageenan (0·1 mL of 1% suspension in 0·9% NaCl), injected below the plantar aponeurosis of the hind paw (Winter et al 1962). The index of inflammation was the increase in the paw volume after the injection of the phlogistic agent. The paw volume, up to the ankle joint, was measured by a mercury plethysmograph, before and at hourly intervals for 4 h, and then at 8 h, 12 h and 24 h after carrageenan administration, and has been expressed in units, each unit representing 1 cm (volume = 0·075 mL) length of displaced mercury. Groups of rats were decapitated, 30 min, 1, 2, 3, 4, 8, 12 and 24 h after the induction of the inflammation, and the brains quickly removed for spectrophotofluorometric estimation of monoamines (Haubrich & Denzer 1975). In a separate

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