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ABSORPTION OF DRUGS FROM THE NASAL MUCOSA OF RAT

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SUMMARY

Absorption of drugs through the nasal mucosa was studied to find out the suitability of the nasal mucosa as a new site of administration. The in situ recirculation test with the nasal cavity of rat showed that absorption of weak electrolytes such as salicylic acid and aminopyrine was highly dependent on the amount of undissociated molecules following the pH partition theory. The in vivo studies on absorption through the nasal mucosa showed that an orally well-absorbed drug such as salicylic acid and bucolome was absorbed comparably to that by injection, while poorly absorbed drugs such as phenol red, sulbenicillin, cefazolin, cephacetrile and insulin, were also absorbed well through the nasal mucosa. These results suggest that the nasal route of administration would be useful as a new route of administration of drugs, to enhance the drug bioavailability.

INTRODUCTION

Absorption of drugs from the gastrointestinal tract, rectum, vagina, oral cavity and lung have been studied by many researchers to correlate the absorption with the physicochemical properties of the drugs.

However, aside from the work on insulin (Hirai et al., 1978) and luteinizing hormone releasing hormone (Fink et al., 1974; Bergquist et al., 1979), close studies on the nasal route for the administration of drugs were quite limited.

The purpose of this study was to establish the in situ and in vivo experimental methodology for the nasal absorption of drugs in rats and to investigate the relationship between the physicochemical properties and the nasal absorption of drugs.

MATERIALS AND METHODS

Materials

Salicylic acid [1], aminopyrine [1], sodium barbital [1], bucolome [2], phenol red [1], sulbenicil-

[1] Wako Pure Chemical Industries, Osaka, Japan.
[2] Takeda Chemical Industries, Osaka, Japan.

lin [2], cefazolin [3], cephacetrile [2] and insulin [4] were obtained from commercially available sources. All other chemicals used were of reagent grade.

Absorption experiment of in situ nasal recirculation

Sprague–Dawley male rats weighing 200–300 g were injected intraperitoneally with 100 mg/kg of sodium phenobarbital and 50 mg/kg of sodium pentobarbital to induce anesthesia and were incised on the neck to expose the trachea. Polyethylene tube (PE-260) [5] was inserted into the trachea. A small incision was also made on the esophagus to insert a polyethylene tube (PE-260) toward the posterior part of the nasal cavity and then the nasopalatine tract leading from the nasal cavity to the oral cavity was closed with an adhesive agent [6] to prevent the drainage of drug solution from the nasal cavity. Connections were made with vinylchloride tubes as shown in Fig. 1 to carry out recirculation of drug solution.

Twenty ml isotonic buffer solution of a drug, prepared by the composition of Kakemi (Kakemi et al., 1965), was recirculated by pump at a rate of 2.5 ml/min at 37°C. To determine the absorption through the nasal mucosa, the residual amount of a drug in the recirculated solution was determined periodically.

Surface pH determination of the nasal mucosa

The surface pH of the nasal mucosa was determined by means of the steady-state method employed by Hogben (Hogben et al., 1959), Schanker (Schanker, 1959) and Kakemi (Kakemi et al., 1965), respectively for the determination of the virtual pH of the small intestine, colon and rectum. The same operation was undergone on rats as the recirculation method under the anesthesia induced by ether. Then sodium barbital of 100 mg/kg dose was injected through the femoral vein. Ten minutes after the intravenous administration, sodium barbital saline solution at a concentration of 0.75 mM (155 μg/ml) was begun to recirculate through the nasal cavity. Concentration of barbital in the recirculated solution was followed every 10 min and the steady-state was confirmed after 30 min of the recirculation. After 50 min, the rats were incised on the abdomen to collect the blood from the inferior vena cava. The blood sample was centrifuged at 3000 rpm for 10 min to obtain the plasma, an aliquot of which was ultrafiltrated through the visking tube [7] under 6000 rpm for 60 min to determine the protein binding of the drug and the rest of the plasma was employed to determine the plasma level of the drug.

The surface pH values of the nasal mucosa were calculated from Eqn. 1 modified the Shore equation (Shore et al., 1957);

$$\frac{C_{nose}}{(1-P)\cdot C_{plasma}} = \frac{1+10^{(pH_{nose}-pK_a)}}{1+10^{(pH_{plasma}-pK_a)}} \quad (1)$$

where C_{nose} is the concentration of barbital in the recirculated solution at steady-state

[3] Fujisawa Pharmaceuticals, Osaka, Japan.
[4] Shimizu Seiyaku, Shizuoka, Japan.
[5] Intramedic PE-260, Clay Adams, New York, U.S.A.
[6] Aron Alpha A, Sankyo, Tokyo, Japan.
[7] Shiraimatsu Instruments, Osaka, Japan.

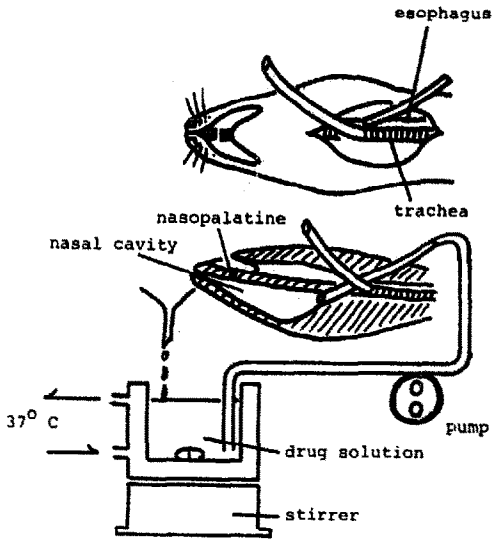

Fig. 1. Apparatus used for recirculation experiments of the rat nasal cavity.

(μg/ml); C_{plasma} is the plasma level of barbital at steady-state (μg/ml); P is fraction of protein binding to barbital in plasma; and pK_a is the pK_a of barbital (7.8).

In vivo absorption through the nasal mucosa

The same operation was performed on rats as that for the in situ recirculation, with the exception that a polyethylene tube inserted into the posterior nasal cavity through the esophagus was closed at the end with cotton and an adhesive agent. Twenty μl of the drug solution was administered to the nasal cavity by means of a micropipette through the nostril, which was closed immediately after the administration with an adhesive agent. Administered drugs were salicylic acid at a dose of 5 mg/rat, bucolome at 10 mg/rat, phenol red at 2 mg/rat, sulbenicillin at 3 mg/rat, cefazolin at 2 mg/rat, cephacetrile at 1 mg/rat and insulin at 20 U/kg. The drugs were dissolved in pH 7.4 buffer, except insulin which was dissolved in pH 3.1 buffer. These solutions were also administered orally, intramuscularly and intravenously for the control studies. The blood samples were taken periodically from the tail vein and urine samples were collected for 4 h after the administration.

Analytical method of drugs

The drugs used in this study were determined according to the following methods. Salicylic acid was determined by the method of Smith (Smith et al., 1946), aminopyrine by the method of Brodie (Brodie and Axelrod, 1950), barbital by the method of Goldbaum (Goldbaum, 1948), bucolome by the method of Mima (Mima et al., 1965) and phenol red by the method of Kakemi (Kakemi et al., 1970). Sulbenicillin, cefazolin and cephacetrile were determined microbiologically by the cup method using *Sarcina lutea* ATCC 9341 as the test organism. The plasma glucose level was determined by the *o*-toluidine method developed by Hyvärinen (Hyvärinen and Nikkilä, 1962).

RESULTS AND DISCUSSION

In situ absorption of salicylic acid and aminopyrine through the nasal mucosa

In situ absorption studies through the nasal mucosa were carried out using lipid-soluble weak electrolytes, such as salicylic acid, aminopyrine, and a highly water-soluble substance, phenol red. Fig. 2 shows the residual amount of the drug in the recirculated solution with time when each 1 mM solution of salicylic acid, aminopyrine and phenol red was recirculated at pH 4.3, 7.9 and 7.4, respectively. Salicylic acid and aminopyrine disappeared following the apparent first-order kinetics from the recirculated solution, although phenol red hardly disappeared within 2 h. The absorption rate constants of salicylic acid and aminopyrine were calculated from the slopes of the drug remaining versus time at several pH values. Fig. 3 shows the relationship between the absorption rate constant and pH of the recirculated solution. For aminopyrine, the absorption rate constants increased at high pH values and the pH profile of the absorption rate constant showed a good coincidence with the theoretical profile of the undissociated fraction versus pH. This suggests that aminopyrine could be absorbed through the nasal mucosa by a process of passive transport of undissociated molecules.

For salicylic acid, it is evident from Fig. 3 that the absorption rate constants decrease at high pH values and that the curve deviates significantly from the pH profile of undissociated fraction. This is explained by the fact that salicylic acid is absorbed through the nasal mucosa even in a pH range where it exists in the form of dissociated molecules. Such phenomena have been reported in the in situ absorption of salicylic acid (Hogben et al., 1959) and sulfonamides (Koizumi et al., 1964) from the small intestine in rats. The absorption of drugs through the nasal mucosa in the in situ method, just like from the gastrointestinal tract, would be affected not only by the undissociated molecules but also by the lipid solubility, the absorption rate of the molecules and the binding to the mucosa (Kakemi et al., 1969).

Fig. 4 shows the absorption of the drugs within 1 h with the concentrations of 0.25, 0.5, 1 and 2 mM in pH 6.4 buffer. It is evident that aminopyrine is absorbed at a constant

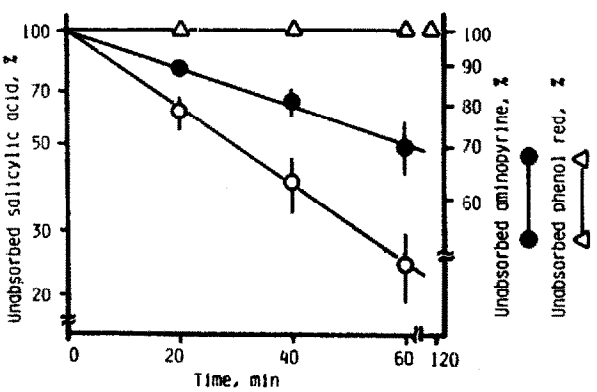

Fig. 2. Disappearance of salicylic acid and aminopyrine through the nasal mucosa of rats. Each value is the mean ± S.E. for 4 animals. ○, 1 mM salicylic acid in pH 4.3 buffer; ●, 1 mM aminopyrine in pH 7.9 buffer; △, 1 mM phenol red in pH 7.4 buffer.

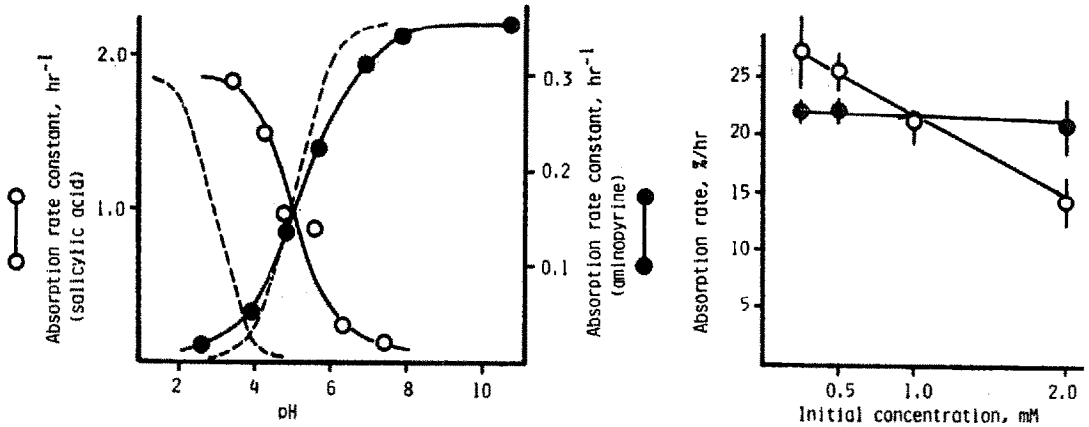

Fig. 3. Absorption rate constants of salicylic acid and aminopyrine through the nasal mucosa of rats at various pHs. Each point is expressed as the mean of 4 animals. ○, 1 mM salicylic acid; ●, 1 mM aminopyrine; – – –, theoretical undissociated fraction of the drugs.

Fig. 4. Absorption rate of salicylic acid and aminopyrine through the nasal mucosa of rats from solutions of various concentrations. Each value is the mean ± S.E. for 4 animals. ○, salicylic acid dissolved in pH 6.4 buffer; ●, aminopyrine dissolved in pH 6.4 buffer.

rate of 20% per hour at various concentrations of the recirculated solution, whereas a tendency was observed in salicylic acid to decrease the absorption rate with high concentration of the solution. This phenomenon might probably be due to the change in the binding of salicylic acid to the nasal mucosa at high drug concentration, since the binding process to the mucosal surface has been confirmed as an important factor of the small intestinal absorption in rats (Kakemi et al., 1969); and/or it might be due to the alteration of the permeability of the nasal mucosa caused by salicylic acid, since salicylate is well known to induce the gastric mucosal damage at high concentration (Cooke, 1976). Further studies would be necessary to elucidate the transport process of the drug through the nasal mucosa.

Surface pH of the nasal mucosa

It is well known that the absorption of weak electrolytes is affected by the surface pH of a biomembrane as well as the pH of the administered solution. Consequently, pH determination of the nasal mucosa was done with barbital as an absorption marker according to the steady-state recirculation method and similarly for the determination of virtual pH on the small intestine, colon and rectum.

Table 1 shows the virtual pH values of the nasal mucosa calculated from Eqn. 1. The mean pH value of the nasal mucosa is 7.39 which is almost the same as that of the plasma. This is probably due to the fact that the nasal mucosa is very thin and has the well developed network of the blood vessels.

In vivo absorption through the nasal mucosa

Salicylic acid and bucolome (Mima et al., 1965), which were lipid-soluble and easily absorbed from the gastrointestinal tract, were applied to the nasal cavity to determine the

TABLE 1

THEORETICAL SURFACE pH OF THE NASAL MUCOSA OBTAINED BY STEADY-STATE EXPERIMENT

Exp.	C_{nose} [a]	C_{plasma} [b]	p [c]	pH_{nose} [d]
1	134.6	131.5	0.176	7.39
2	159.6	175.3	0.234	7.35
3	143.4	176.9	0.182	7.40
4	137.6	173.8	0.139	7.42
mean				7.39

[a] Concentration of barbital in the recirculated solution at steady-state (μg/ml).
[b] Plasma level of barbital at steady-state (μg/ml).
[c] Protein binding in plasma.
[d] Surface pH of the nasal mucosa.

absorption of the drug through the nasal mucosa, while they were also administered orally and intramuscularly for the control studies. Fig. 5 shows the time course of plasma levels of salicylic acid and bucolome after the administration from various routes in rats. Plasma levels of the drugs increased rapidly and showed the maximum 15 min after the

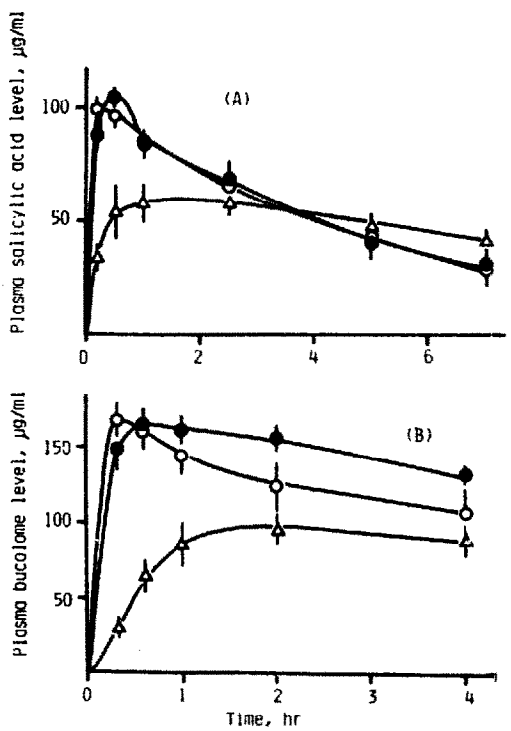

Fig. 5. Mean plasma levels of salicylic acid and bucolome after the administration by various routes in rats. Each value is the mean ± S.E. for 3 animals. A: salicylic acid (5 mg/rat). B: bucolome (10 mg/rat). ○, nasal; △, oral; ●, intramuscular administration.

nasal administration. This suggests that lipid-soluble drugs are rapidly absorbed from the nasal cavity and that quicker action can be expected by the nasal administration.

It is well known that phenol red (Schanker, 1959), sulbenicillin (Bergan, 1978), cephacetrile and cefazolin (Brogard et al., 1978) are poorly absorbed from the gastrointestinal tract because of their highly water-soluble and lack of lipophilic properties. Fig. 6 shows the urinary recoveries of the drugs within 4 h after the nasal administration, compared with those in the oral and intramuscular administration. After the oral administration, poor absorption was confirmed for the drugs; however, after the nasal administration, the per cent dose excreted in urine was nearly one-half of that after intramuscular administration. Also, phenol red which was hardly absorbed through the nasal mucosa in the in situ recirculation method, was well absorbed in the in vivo studies. These results suggest that even poorly absorbed drugs are easily absorbed after the nasal administration in the in vivo studies. The different absorption between in situ and in vivo studies is probably due to the experimental conditions, e.g. in the in situ experiment, the volume of the rat nasal cavity (0.2–0.3 ml) is extremely small as compared with that of the recirculated solution (20 ml).

Polypeptides, being high molecular weights and hydrolyzed by the enzymes in the gastrointestinal mucosa, are hardly absorbed without any metabolic change when given orally. The absorption of insulin through the nasal mucosa was investigated in rats by determining the blood glucose levels periodically after the administration, while control studies were carried out by oral and intravenous administration at a dose of 100 U/kg and 2 U/kg, respectively. As shown in Fig. 7, the plasma glucose level scarcely decreased at a high dose after the oral administration; however, they decreased significantly even at a relatively low dose after the nasal administration. For the nasal administration of insulin, about 10 times the dose in the intravenous administration is necessary to obtain an equal pharmacological effect.

On the transport process of a drug through the nasal mucosa, partition of the drug to the membrane was found to be one of the important factors from the results of the in

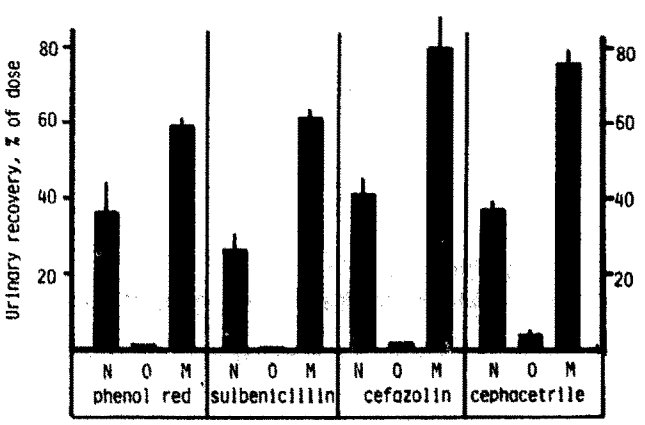

Fig. 6. Urinary recoveries of phenol red (2 mg/rat), sulbenicillin (3 mg/rat), cefazolin (2 mg/rat) and cephacetrile (1 mg/rat) within 4 h after the administration through various routes in rats. Each value is the mean ± S.E. for 4–7 animals. N, nasal; O, oral; M, intramuscular administration.

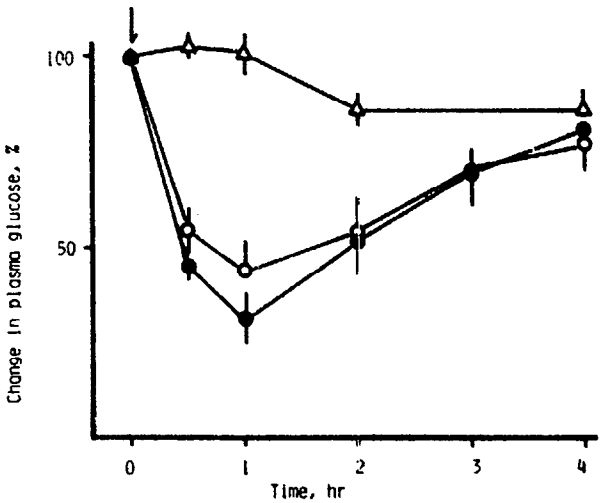

Fig. 7. Change in plasma glucose level after the administration of insulin through various routes in rats. Each value is the mean ± S.E. for 5 animals. ○, nasal 20 U/kg; △, oral 100 U/kg; ●, intravenous 2 U/kg.

situ recirculation studies on lipophilic drugs and the fact that highly water-soluble drugs were also absorbed well through the nasal mucosa in the in vivo studies suggested that the drugs would transfer through the pores or by some other non-passive process into the blood stream. In order to evaluate the absorption of a drug through the nasal mucosa, an in vivo absorption study seems to be preferable to the in situ recirculation method.

Thus, it becomes evident that the nasal cavity is a useful application site for both lipid-soluble and water-soluble, poorly absorbed drugs including polypeptides, and that the nasal route is superior to the oral route, especially for poorly absorbed drugs, from the standpoint of the bioavailability.

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