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Effects of Tonicities and Solutes of Solutions on the Lethality and the Survival Time after Intraduodenal Administration of Ephedrine Hydrochloride in Mice

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Attempts confirming the effect of the transmucosal fluid movement and the glucose effect on the intestinal drug absorption, those of which had been revealed in the previous studies of *in situ* experiment, in the *in vivo* studies were undertaken using an intraduodenal administration method to mice. These effects were reflected amply on the indices of the lethality and the survival time after the administration of ephedrine hydrochloride.

From the results obtained in the present study, it was able to be concluded that the effects of the transmucosal fluid movement and the glucose are observed realistically in the *in vivo* experiment. In the course of the studies, calcium chloride was appeared to affect similar in nature to the glucose effect on the drug absorption from the small intestine.

Studies concerning the effect of the transmucosal fluid movement on drug absorption from rat small intestine using the method of recirculating perfusion[2] with test solution having different tonicities of sodium chloride and glucose have been reported from our laboratories.[3] The results obtained revealed that the absorption of drugs increased with decreasing tonicity of the perfusion solutions in both cases of the solutes which were applied to adjust the osmolarity of the perfusates. In the course of the study it was found out that glucose played peculiar effects on the absorption of ionized forms of drugs, which was not explainable by and was different in nature to the transmucosal fluid movement, and such a phenomenon was termed arbitrarily the glucose effect for the sake of convenience in the previous study.[3]

Before driving our investigations to further advances, it should be necessary to confirm that the above evidences which had been revealed in the *in situ* experiment are also able to be observed in an *in vivo* experiment.

To achieve the purpose, an adequate experimentation of *in vivo* study should be undertaken and, moreover, indices of the absorption of drug should be expressed by a competent pharmacological effect on the animal. Introduction of ephedrine hydrochloride into our investigations was made with the lethal dose to mice and an intraduodenal instillation route of administration was employed in the present study.

Experimental

Materials——Ephedrine hydrochloride used was of JP VIII grade and all other chemicals used in the study were of reagent grade and obtained from commercial sources.

Preparations of the Test Solutions——The concentration of ephedrine hydrochloride in all of the test solutions was decided based on the oral LD_{50} of ephedrine in mice. The solutes used to adjust tonicities of the solutions were sodium chloride, glucose and calcium chloride. The compositions and their amounts in each test solution are listed in Table I.

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2) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. & Exper. Therap.*, **123**, 81 (1958).
3) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.*, **23**, 1856 (1975).

Table I. Compositions of Ephedrine Hydrochloride Solutions for the Intraduodenal Administrations to Mice

Solutions	NaCl isotonic	NaCl hypotonic	Glucose isotonic	$CaCl_2$ isotonic
Tonicity[a)]	1	1/2	1	1
Ephedrine Hydrochloride (mg)	1000	1000	1000	1000
Solute	NaCl	NaCl	Glucose	$CaCl_2$
Amount of solute Added (mg)	620	170	3900	1100
Distilled water	distilled water was added to make 100 ml			

a) Tonicity of 0.9% sodium chloride solution is regarded as 1 in the measuring the osmolarities of the solutions using Hitachi-Perkin Elmer Molecular Weight Apparatus of Model 115

Taking the sodium chloride equivalence of the drug in 0.30[4)] into considerations, the amount of the solutes added was calculated and verifications of the resulted osmolarities of the solutions were conducted using Hitachi Perkin-Elmer Molecular weight Apparatus of Model 115 with the method developed by Kitazawa and Komuro.[5)] However, osmolarities of the solutions instillated to the control group of the animal were adjusted to isotonic or a half of isotonic of respective solutions only by each solute.

Procedures of Intraduodenal Instillation——Male ddY strain mice of weighing 12 to 18 g were used after an overnight fasting, but were allowed free access to water until the time of the experimentation. Mice were randomly separated into two groups. One group which was nominated as an experimental group was administered the test solution and another group which was nominated as a control group was instillated the solution without the drug. The animals were anesthetized slightly with ether, and placed and fixed on back on an operating table in an usual manner. Following removement of abdominal hair, the duodenum was exposed by a midline incision. To avoid a backflowing of the instillation solution into stomach, the test solution was instillated with cautions into proximal end of the duodenum to a direction of distal small intestine using a needle of No. 5 gauge which was equipped to an injection syringe having a volume of 1.5 ml.

After the instillation and confirming that any drops of the test solution did not spurt out of the hole through which the instillation had been conducted, the incision was immediately closed by ligatures and small amount of 2% of mercurochrom solution was applied to the incised part to avoid any infections. Further anesthetization, if it was necessary during the operating procedures, was applied at any time with ether, but the anesthesia should be lasted few minutes after the operating procedures.

Both groups of mice were kept individually until the time of onset of the pharmacological effects of the drug. All the mice recovered from anesthesia few minutes after the procedures and behaved normally, and discrimination of the animals between the experimental group and the control group was difficult at this stage of the experiment. However, after elapsing a certain period, sudden deaths were occurred on about a half of the experimental group of mice following tumblings and short periods of severe convulsions. Concerning the another half of the experimental group, the mice also tumbled and slight convulsions delayed for almost an hour, but these symptoms were ceased gradually and the mice recovered normal within two hours after the instillation.

Measurements of the survival time which indicates the period from the instillation to the decease and the lethality which indicates percent of the animals deceased in the experimental group were conducted without any difficulties.

Results

Determination of the Volume of the Solution Instillated

Before begining a series of experiments, the dose in volume of the drug solution having fixed concentration of 10 mg/ml should be determined. Assuming that both the lethality and the survival time would increase or decrease when the tonicities and the solutes of the instillation solution were varied from isotonic sodium chloride to others, the dose which gave approximate 50% of the lethality and more than 20 minutes of the survival time of the solution of isotonic saline containing the drug would be desirable. The dose was surveyed with changing the volume of the solution.

4) A.N. Martin, "Physical Pharmacy," Lea & Febiger, Philadelphia, 1960, p. 292.
5) S. Kitazawa and T. Komuro, *Hitachi Scientific Instrument News*, **15**, 1143 (1972).

An aliquot volume of the instillation was determined to be 1.3 ml per 20 g of body weight of the subject on the basis of the results which are presented in Table II.

Table II. Effect of Dose in Volume of NaCl Isotonic Solution[a] on the Mortality and the Survival Time of the Mice after the Intraduodenal Instillation

Dose[b] (ml/20g of body Wt.)	n[c]	Average mortality (%)	Average survival time (min)
1.2	7	42.9	33.3
1.3	21	52.9	26.9

a) The compositions of this solution are shown in Table I.
b) The concentration of ephedrine hydrochloride is 10 mg/ml.
c) numbers of the experiments

Effects of Tonicities and Solutes on the Lethality and the Survival Time in Mice

The results obtained after the intraduodenal instillation of ephedrine hydrochloride solution having different tonicities and solutes are summarized in Figure.

As illustrated in the Figure, the lethality of the animal increased to the average of 69.2% and the survival time was decreased to the average of 15.3 minutes, which was significantly different, when the drug solution having a half of the isotonicity of sodium chloride, while the lethality and the survival time of isotonic sodium chloride containing the drug showed 52.4% and 26.5 minutes respectively.

More apparent differences were observed when glucose was existed with the drug in an isotonic solution, the lethality increased to 80.0% and the survival time was shortened to 9.2 minutes, which was also significantly different, respectively on the average.

The results obtained with isotonic calcium chloride showed that both indices varied from those of isotonic sodium chloride to 62.5% and 15.6 minutes respectively on the average.

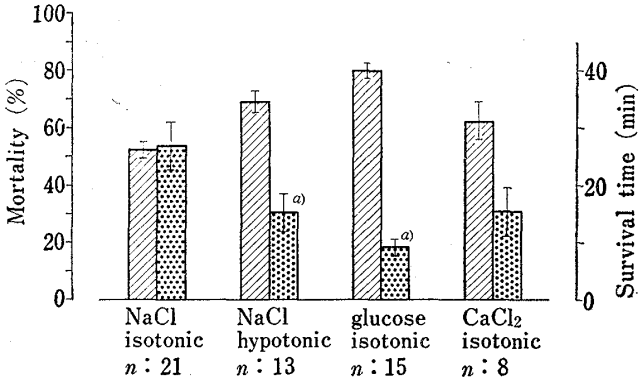

Fig. Effect of Tonicity and Solute on the Mortality and the Survival Time Following the Intraduodenal Administration of Ephedrine Hydrochloride in Mice

The results represent the mean of numbers of experiments indicated under each histogram, and vertical bars show standard errors of the mean.
key: ▨: mortality, ▩: survival time,
a) indicates significant difference (<0.05) to the respective result of the NaCl isotonic. Calculations followed the student t-test.

Attentions should be focussed on the differences observed in both indices while the solutions had the same tonicities in all cases of sodium chloride, glucose and calcium chloride, and while the solutions had the same solute of sodium chloride with different tonicities.

Discussion

Validity of Using Ephedrine Hydrochloride in the Study

Making a proper choice of drugs is a key of obtaining more suitable results. Ephedrine hydrochloride was selected in this study of *in vivo* experiment. Many reasons were considered in the selections, main of them are the following.

As pK_a of the drug is 9.4,[6] the dissociated form of this drug in pH 6.5 which had been considered as physiological pH of the small intestine[3,7] must be a cationic form. This is a suitable characteristic for expecting the glucose effect *in vivo* study. In fact, the absorption of ephedrine hydrochloride from rat small intestine was enhanced when the solute of the perfusate was glucose in place of sodium chloride and apparent two separate regression lines indicating the glucose effect were obtained in the *in situ* experiment.[8]

Molecular weight of the drug is 201.7, and this molecular weight falls in between those of sulfanilamide (mole wt.: 172.21) and metoclopramide (mole wt.: 299.81). The absorption of these drugs has been previously demonstrated to be affected by the transmucosal fluid movement.[3]

Although the drug has many kinds of pharmacological efficacies corresponding to blood concentrations in animals,[9] an excessive high concentration causes decease on animal, which is competent pharmacological responce as a more distinct marker of absorption of the drug. Oral LD_{50} of ephedrine is known as 400 mg/kg in mice, and one gram of the drug is soluble in 3 ml of water.[10] These data are considered to be adequate in determining the instillation volume to duodenum of the animal, which will be mentioned in the following paragraph.

Intraduodenal Instillation and the Volume of the Solution

Studying the drug absorption from the intestine *in vivo*, an oral route of administration has often been employed. However, this route of administration would be improper in the investigations of effect of the transmucosal fluid movement on drug absorption in more detail and, moreover, on the time of the onset of pharmacological effect on the animal, because there would be a number of factors influencing the results such as gastric emptying, and dilutions of the drug solution with digestive fluids in the stomach.[11] Intraduodenal instillation method was employed in our investigations to exclude these factors as possible as in the analysis of the results.

The method seemed to impose some restrictions on the volume of the solution instillated to mice. Too large volume was found to be excluded, because it caused expansions of duodenal part and the solution often spurted out through a hole through which the solution had been instillated, and the front of the solution reached almost the distal end of the ileum of the animal. It would be desirable to keep the whole volume of the solution in the duodenal and the proximal half of the jejunal part of the intestine at the time of ceasing the instillation. From an anatomical observation, the volume of the solution should be less than 1.5 ml.

In considering LD_{50} of the drug, the concentration of the solution was determined to be 10 mg/ml to keep the instillation volume less than 1.5 ml. After searching for a proper volume which caused approximate 50% of the lethality and more than 20 minutes of the survival time with isotonic saline containing the drug, the volume was decided to be 1.3 ml, which is satisfactory to promote the studies. To exclude the effect of the volume of the instillation solution,[12] this volume was kept in all cases of the present studies.

Effect of Tonicity of the Instillation Solution

Increase in the lethality and decrease in the survival time of the animal were observed when the tonicity of the instillation solution was decreased to a half of the isotonic sodium chloride. These evidences suggested that the absorption of ephedrine hydrochloride from the

6) A.N. Martin, "Physical Pharmacy," Lea & Febiger, Philadelphia, 1960, p. 219.
7) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
8) S. Kitazawa, H. Ito, and I. Johno, "in preparation."
9) J.H. Gaddum and H. Kwitokowski, *J. Physiol.*, **94**, 87 (1938).
10) "Merck Index" 8th ed., edited by Merck & Co., Rahway, 1968.
11) H.W. Davenport, "Physiology of the Digestive Tract," Year Book Medical Publisher, Chicago, 1971, p. 47.
12) G. Vogel and J. Grundei, *Arzneim.-Forsch.*, **21**, 515 (1971).

intestine increased with decreasing tonicity of the instillation solution. The results obtained in the separate studies in our laboratories[13] indicated that differences in the transit of solutions having different tonicities in the small intestine of this animal were observed and the transit time of solutions of a half of the isotonicity delayed as half as that of the isotonic solutions after a certain period of the instillation in the same manner as employed in this study. This evidence suggested that the surface area of the small intestine that faced to the instillation solution would decreased in the cases of the hypotonic solution. Although the decreasing in the surface area through which the drug would be absorbed might occur in this experiment, the evidence that the total amount of the drug absorbed would increase in the cases of instillation of the hypotonic solutions might suggest that the drug absorption through a unit square of the small intestine would increase as compared to the cases of isotonic solutions.

These lines of evidences and estimations were strongly supported by the results in the previous report[3] indicating that the absorptions of drugs increased with increasing of the transmucosal fluid movement directing from the lumen to blood stream, which had been occurred in cases of the perfusion solutions of reduced osmolarities.

The results obtained in these *in vivo* studies encouraged the authors in drawing the following conclusions that the results concerning the relation of the drug absorption to the transmucosal fluid movement which had been obtained in the *in situ* perfusion studies were evidently demonstrated also *in vivo* studies, and that the increase in lethality and decrease in the survival time were the result of the increasing of the drug absorption due to increasing the absorption of the fluids in the intestinal tract.

Effect of Glucose in the Instillation Solution

As has been indicated in the Figure, remarkable increase (more than 1.5 times) in the lethality and significant decrease (less than one third) in the survival time of the mice were marked in cases of the instillation of isotonic glucose solution in place of isotonic sodium chloride solution. These results might suggest that the total amount of the drug absorbed from the isotonic glucose solution was much more than that of from the isotonic and also from the hypotonic sodium chloride solutions.

McHardy and Parsons,[14] and Parsons, *et al.*[15] revealed that glucose facilitated the transmucosal fluid movement. This, of course, might cause increase in the drug absorption. In the previous report from our laboratories,[3] it was made clear that fluid movement brought about in the presence of glucose was not remarkably different compared to the corresponding osmolarity of the sodium chloride perfusate. This finding suggested that the difference in osmolarity of the perfusate was considered to affect always dominantly than the difference in component of the perfusate on the transmucosal fluid movement in the *in situ* experiment. However, the result obtained in the present study revealed that the fluid movement brought about by the isotonic glucose exceeded that of brought about by sodium chloride which had hypotonic osmolarity, that was a half of an isotonicity. Such a phenomenon might suggest the possibility that an extent of the glucose effect would be exaggrated *in vivo* experiment than that of *in situ* experiment.

Thus, it appears difficult to explain the increased absorption of the drug without the glucose effect which had been originally observed in the case of a cationic drug in the previous study.[3] Ephedrine hydrochloride meets every condition suffering the glucose effect in the intestinal absorption and, in fact, had been revealed in the separate study[8] to be suffered the glucose effect in the *in situ* perfusion study with rat small intestine as mentioned above.

13) S. Kitazawa and I. Johno, "in prepararation."
14) G.T.R. McHardy and D.S. Parsons, *Quart. J. Physiol.*, 42, 33 (1957).
15) B.J. Parsons, D.H. Smyth, and C.B. Taylor, *J. Physiol.*, **144**, 387 (1958).

These evidences made it possible to conclude that the increased pharmacological effects observed in the presence of glucose in the instillation solution should be due to the glucose effect and that the effect is clearly demonstrated in such an *in vivo* study.

Effect of Other Salt in the Instillation Solution

Several reports[16] in the literature suggested that various salts may alter the transmucosal fluid movement, calcium chloride, as one of the examples, was introduced as the solute in the instillation solution of isotonic tonicity. An inhibitory effect of this salt on the absorption of tetracycline was reported by Tonelli, *et al.*[17] and Kakemi, *et al.*[18]

The pharmacological effects obtained showed somewhat different from that of the isotonic sodium chloride solution and suggested calcium chloride might have a similar effect in nature as that of glucose in the drug absorption from the small intestine, as far as the drug has no specific characteristics as that of tetracycline. However, the effect is not so remarkable as that of glucose and, of course, the data obtained so far are not enough to conclude that calcium chloride may alter the transmucosal fluid movement. More efforts should be undertaken to reveal more definitely the effect of calcium chloride on the absorption of drugs from the small intestine of the animal.

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16) J.S. Fordtran and T.W. Locklear, *Am. J. Digest. Diseas.*, **11**, 503 (1966); M.D. Lifshitz, J.A. Garcia, and L.E. Earley, *Kidney International*, **4**, 362 (1973).
17) G. Tonelli, L. Alfano, and E. Takesue, *J. Pharmacol.*, **145**, 386 (1964).
18) K. Kakemi, H. Sezaki, H. Ogata, and T. Nadai, *Chem. Pharm. Bull.* (Tokyo), **16**, 2200 (1968); K. Kakemi, H. Sezaki, H. Hayashi, and T. Nadai, *Chem. Pharm. Bull.* (Tokyo), **16**, 2206 (1968).