Involvement of Active Sodium Transport in the Rectal Absorption of Gentamicin Sulfate in the Presence and Absence of Absorption-Promoting Adjuvants

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Abstract □ The involvement of active sodium transport in the rectal absorption of gentamicin sulfate was examined in rats, employing aqueous microenemas of known total ionic strength (μ) in the presence or absence of absorption-promoting adjuvants. Rectal gentamicin bioavailability, which is negligible (1 ± 1.2%) at an ionic strength of 0.15 without adjuvants, is significantly ($p < 0.01$) increased by including adjuvants in the formulation (sodium salicylate, 12 ± 4.0%; sodium-5-bromosalicylate, 59 ± 15.1%; disodium ethylene(dinitrilo)tetraacetate, 24 ± 9.3%). Pretreating the rectal mucosa cells with ouabain, a specific inhibitor of active sodium transport, significantly ($p < 0.01$) reduced gentamicin absorption in response to all three adjuvants. In contrast to previous findings with sodium chloride, high ionic strength choline chloride (μ = 1.056) did not promote gentamicin absorption. The data indicate that active sodium transport is an integral component of rectal absorption of water-soluble compounds and may be involved in the mechanism of action of absorption-promoting adjuvants.

Keyphrases □ Gentamicin sulfate—rectal absorption, effect of choline chloride and ouabain □ Choline chloride—effect on rectal absorption of gentamicin sulfate adjuvant enhancement □ Ouabain—effect on the rectal absorption of gentamicin sulfate □ Rectal absorption—gentamicin sulfate, effect of choline chloride and ouabain.

Normal rectal mucosa is capable of transporting sodium chloride and water from the luminal to the serosal side of the epithelial barrier (1). The bidirectional movement of sodium ions is regulated by both passive and active components, and has been shown to be directly correlated with the rectal transluminal potential difference (2). Previous work in this laboratory has shown that increasing the ionic strength in a microenema containing gentamicin, a water-soluble antibiotic, significantly enhanced the rectal absorption of the drug (3). The enhancing effect due to high ionic strength appeared to be additive to the enhanced rectal absorption effected by sodium salicylate, a proven absorption adjuvant (4, 5).

Two factors in a previous study on ionic strength (3) suggested that there was a possible specific involvement of sodium transport in rectal gentamicin delivery, including delivery which was adjuvant (sodium salicylate) assisted. In the absence of sodium salicylate, gentamicin absorption (at ionic strength of μ = 1.054) was twofold greater when sodium chloride, rather than potassium chloride, was the major contributor to total ionic strength. The addition of sodium salicylate to both sodium chloride and potassium chloride microenemas significantly increased rectal gentamicin absorption, but the same twofold difference in efficacy between sodium chloride and potassium chloride was still observed.

In the current study, the possible involvement of active sodium transport was further investigated by employing two agents, ouabain and choline chloride, which alter electrogenic sodium transport. Ouabain is a metabolic inhibitor which specifically blocks the membrane sodium–potassium pump. Choline chloride is used as a sodium chloride substitute since equivalent ionic strength can be established; but choline itself is not transported by the sodium–potassium pump. The use of these agents has provided data suggesting a direct involvement of active sodium transport in the rectal absorption of gentamicin. The results obtained here may also be of general applicability to other water-soluble compounds whose rectal absorption is enhanced by salicylate-type adjuvants (5–7).

EXPERIMENTAL

Adult male Sprague-Dawley rats (200–250 g) were fasted, with free access to water, for 18–24 hr prior to the experiment. Animals were anesthetized with intramuscular injections of urethane (0.5 ml of 43% ethylcarbamate[1] in distilled water per 100 g of body weight).

Aqueous microenemas (250 μl, pH 5) containing 2.5 mg of gentamicin sulfate[1] were administered with a 1-ml syringe at an intrarectal depth of 2.5 cm. Ionic species (sodium chloride, potassium chloride, or choline chloride) and/or absorption adjuvant were included in the microenemas as indicated in the text and legends. In one set of experiments, sodium-free microenemas were prepared by dissolving gentamicin sulfate and salicylic acid in 50-m*M* tromethamine hydrochloric acid (Tris-HCl) buffer. In experiments employing ouabain, animals received a 250-μl microenema of 25 m*M* ouabain either 15 or 30 min prior to administration of the gentamicin formulation. The gentamicin formulation also contained 25-m*M* ouabain in these experiments. Total ionic strength and percent bioavailability were calculated as described previously (3).

RESULTS

Rectal absorption of gentamicin is negligible (1 ± 1.2%, $n = 3$) when the drug is administered in an aqueous microenema at μ = 0.15 and without an adjuvant. Gentamicin absorption was significantly increased by the addition of 20 mg/kg of sodium salicylate (12 ± 4.0%, $n = 6$, $p < 0.01$), disodium ethylene(dinitrilo)tetraacetate (24 ± 9.3%, $n = 3$, $p < 0.01$), or sodium-5-bromosalicylate (59 ± 15.1%, $n = 3$, $p < 0.01$).

Increasing the microenema ionic strength by the addition of sodium chloride or potassium chloride was previously shown to significantly increase rectal gentamicin absorption (3). Data for choline chloride are shown in Fig. 1. Gentamicin bioavailability in the presence of 1.0 *M* choline chloride (μ = 1.054) was 6 ± 1.0% ($n = 3$) with no significant differences at other choline chloride concentrations (μ = 0.304–0.754). Compared with previous data on increasing ionic strength, the relative order of effectiveness in promoting gentamicin absorption was sodium chloride > potassium chloride > choline chloride (Fig. 1A). The incorporation of sodium salicylate into the choline chloride microenema resulted in gentamicin bioavailability greater than that seen with choline chloride alone (Fig. 1B). When the ionic contributions of sodium salicylate were included in calculations of total ionic strength and the results presented as bioavailability *versus* total ionic strength (3) (Fig. 1B), the profiles with salicylate demonstrated higher bioavailabilities and were approximately parallel to the ionic profiles with the salts alone.

Ouabain pretreatment significantly ($p < 0.01$) decreased gentamicin bioavailability in response to sodium salicylate, sodium-5-bromosalicylate, and disodium ethylene(dinitrilo)tetraacetate (Fig. 2). The effect was more pronounced following the 30-min pretreatment. Even though 30-min ouabain pretreatment significantly reduced bioavailabilities, the

[1] Sigma Chemical Co., St. Louis, Mo.

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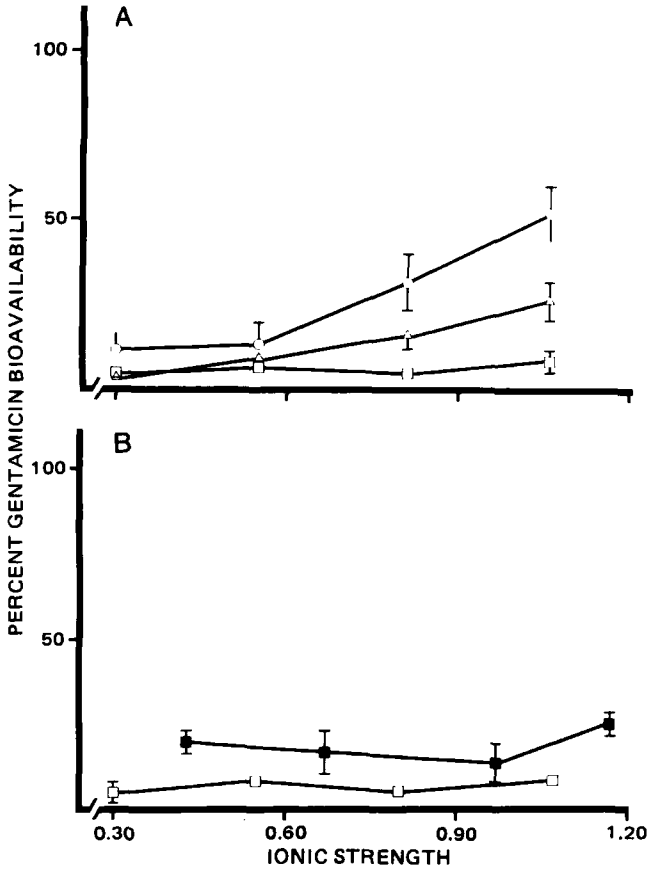

Figure 1—*Gentamicin bioavailability in the presence of various ionic strengths (μ) of sodium chloride (○), potassium chloride (△), and choline chloride (□), choline chloride–sodium salicylate (■). Panel A shows ionic strength profiles for each of the three salts in the absence of sodium salicylate. Panel B shows results of ionic strength profiles with choline chloride in the presence and absence of sodium salicylate. Error bars represent the standard deviation for* n = *3–6 experiments. Where error bars are not shown, the standard deviation is within the dimensions of the symbol.*

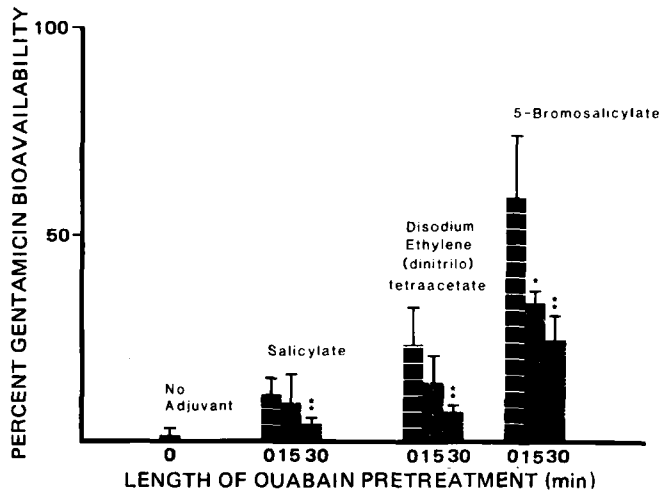

Figure 2—*Effect of ouabain pretreatment on gentamicin bioavailability in response to sodium salicylate, disodium ethylene(dinitrilo)tetraacetate, and sodium-5-bromosalicylate. Gentamicin bioavailability achieved with 20 mg of adjuvant/kg of body weight is represented as 0-min ouabain pretreatment. Histogram bars designated 15 and 30 represent gentamicin bioavailability observed in response to adjuvant activity following a 15 or 30-min exposure of the rectal tissue to 25 m*M *ouabain. Error bars represent the standard deviation for* n = *3–6 experiments. Key: (*)* $p < 0.025$, *(**)* $p < 0.01$.

levels of drug absorption were still greater ($p < 0.05$) than those observed with control microenemas (no adjuvant).

The effect of ouabain pretreatment on salicylate-assisted, high ionic strength rectal gentamicin absorption was examined using microenemas containing 0.5 *M* NaCl. Without ouabain pretreatment, gentamicin bioavailability in the presence of sodium chloride and 20 mg of sodium salicylate/kg body weight ($\mu = 0.574$) was 47 ± 7.1% ($n = 3$). Following a 30-min ouabain pretreatment, bioavailability was reduced to 22 ± 4.4% ($n = 3$, $p < 0.001$).

Sodium-free microenemas, prepared using salicylic acid and nonsodium buffering salts, resulted in gentamicin bioavailability of 9 ± 0.3% ($n = 3$), which was significantly ($p < 0.001$) greater than adjuvant-free control microenemas (1 ± 1.2%, $n = 3$).

DISCUSSION

Previous work in this laboratory suggested the possible involvement of monovalent cation specificity in the rectal delivery of gentamicin, a water-soluble antibiotic (3). Ionic strength profiles demonstrated that sodium chloride afforded higher levels of gentamicin absorption than potassium chloride. The absorption-promoting activity of sodium salicylate appeared to be additive to the enhancing effects of ionic strength.

The possible involvement of active sodium transport was further investigated in this study. Choline chloride, a nontransported ionic equivalent of sodium chloride, and ouabain, a specific inhibitor of the membrane sodium-potassium pump, were tested for effects on rectal gentamicin absorption.

Increasing the microenema ionic strength by the addition of sodium chloride, potassium chloride, or choline chloride demonstrated a preferential response in bioavailability to sodium ions. The inability of high ionic strength choline chloride ($\mu = 1.054$) to promote gentamicin absorption indicated that ionic strength alone was not a major modulator of rectal absorption.

Involvement of the membrane sodium-potassium transport system was further verified in experiments employing ouabain pretreatment of the rectal mucosal cells. The ability of ouabain to significantly reduce gentamicin bioavailability in response to all three adjuvants [salicylate, disodium ethylene(dinitrilo)tetraacetate, 5-bromosalicylate], at normal and elevated sodium chloride ionic strengths, indicates an important influence of active sodium transport in rectal absorption. After 30-min ouabain pretreatment, the level of gentamicin absorption was still significantly greater than the bioavailability observed in experiments with untreated animals given microenemas without adjuvant. One possible explanation for this is that at least a fraction of the adjuvant-enhanced absorption is independent of active sodium transport, assuming that a total inhibition of sodium transport would occur in response to 25 m*M* ouabain pretreatment (not verified by measurements of sodium transport). An alternative explanation is that the ouabain concentration or time of membrane exposure to ouabain may have been insufficient to totally suppress membrane sodium transport. In either case, the definite involvement of active sodium transport in rectal gentamicin absorption has been demonstrated.

The ability of salicylic acid to promote rectal gentamicin absorption when administered as a sodium-free microenema suggests that sodium may not be absolutely required for this delivery system. However, sufficient sodium may normally be present in the fluids of the rectal compartment to permit salicylate-enhanced gentamicin absorption.

The mechanism(s) by which active sodium transport affects rectal absorption of water-soluble compounds is unknown, although the direction and magnitude of fluid movement is significantly affected by the sodium gradient. Regardless of the exact mechanism(s) involved, it is clear that active sodium transport is intimately related to rectal absorption systems. The applicability of these findings to the further development of traditional dosage forms (*e.g.*, suppositories) remains to be demonstrated. However, the regulation of sodium transport may provide a means to predictably alter the permeability of the rectal mucosal cell barrier and to more carefully control adjuvant-enhanced absorption of water-soluble compounds.

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Synthesis and Anticonvulsant Screening of 3,3-Diphenyl-2-pyrrolidone Derivatives

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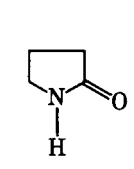
Received March 3, 1982, from the *Chemistry and Life Sciences Group, Research Triangle Institute, Research Triangle Park, NC 27709.* Accepted for publication June 7, 1982.

Abstract □ Six derivatives of 3,3-diphenyl-2-pyrrolidone were synthesized and screened for anticonvulsant activity. The synthetic route involved a mono-*N*-demethylation of an intermediate *N*,*N*-dimethylaminonitrile with methyl chloroformate followed by cleavage of the carbamate group. Of the six derivatives, (±)-2-imino-1,5-dimethyl-3,3-diphenylpyrrolidine hydrochloride was effective in protecting mice against maximal electroshock (MES) -induced seizures at a 30-mg/kg dose level.

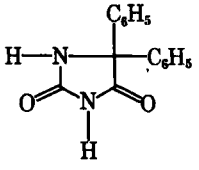
Keyphrases □ Anticonvulsant screening—synthesis of 3,3-diphenyl-2-pyrrolidone derivatives, maximal electroshock-induced seizures, mice □ 3,3-Diphenyl-2-pyrrolidone—synthesis and anticonvulsant screening, derivatives, maximal electroshock-induced seizures, mice

The reported (1) anticonvulsant activity of 2-pyrrolidone (I), the lactam of γ-aminobutyric acid, stimulated interest in 2-pyrrolidone derivatives as potential anticonvulsants. As it incorporated several features of the anticonvulsant diphenylhydantoin (II), the 3,3-diphenyl-2-pyrrolidone (III) system seemed a promising one for the study. When tested in rats, 3,3-diphenyl-2-pyrrolidone (III) itself was less effective than (±)-3-ethyl-3-phenyl-2-pyrrolidone against both pentylenetetrazol[1] and electrically-induced convulsions (2). Somewhat earlier, it was observed (3) that the structurally related α,α-diphenylsuccinimide (IVa) and its *N*-methyl derivative (IVb) ranked first and eighth in a series of 39 succinimides in protecting mice against electrically induced convulsions.

The work on 3,3-disubstituted-2-pyrrolidones (2) and α-phenylsuccinimides (3) showed that the presence of an asymmetric center at the 3-position often enhanced anticonvulsant activity. The present study was undertaken to determine if the introduction of an asymmetric center at the 4- or 5-position of the 3,3-diphenyl-2-pyrrolidone (III) system would have a similar beneficial effect. Accordingly, (±)-1,4-dimethyl-3,3-diphenyl-2-pyrrolidone (Va) and (±)-1,5-dimethyl-3,3-diphenyl-2-pyrrolidone (VIa) were synthesized and evaluated for anticonvulsant activity. The corresponding amidines, Vb and VIb, were also prepared to compare them with the neutral lactams. Moreover, the amidine functionality was expected to provide a handle for further structural modification as exemplified by the acyl derivatives, VIc and VId. The test compounds were prepared using modifications of previously reported (4–6) procedures.



I



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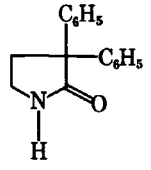
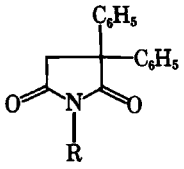
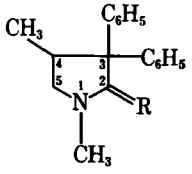


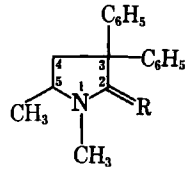
III



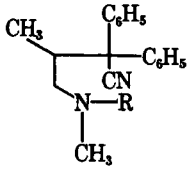
IVa: R = H
IVb: R = CH_3



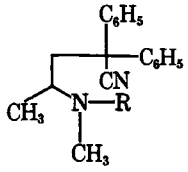
Va: R = O
Vb: R = NH



VIa: R = O
VIb: R = NH
VIc: R = $NCOCH_3$
VId: R = NCO_2CH_3



VIIa: R = CH_3
VIIb: R = CO_2CH_3



VIIIa: R = CH_3
VIIIb: R = CO_2CH_3

RESULTS AND DISCUSSION

Because of the interest in amidines Vb and VIb, the synthetic route (4–6) involving them as intermediates was preferred to other procedures (7, 8) for preparing (±)-1,4-dimethyl-3,3-diphenyl-2-pyrrolidone (Va) and (±)-1,5-dimethyl-3,3-diphenyl-2-pyrrolidone (VIa). The mono-*N*-demethylation of the *N*,*N*-dimethylaminonitriles VIIa and VIIIa was readily effected using excess methyl chloroformate. The subsequent conversion of carbamate VIIIb to VIb hydrochloride was carried out in refluxing hydrochloric acid. In contrast, carbamate VIIb failed to either dissolve or react in hydrochloric acid. Moreover, the compound was recovered essentially unchanged after 17 days of reflux in ethanol–hydrochloric acid (2:1). Ultimately, the transformation of VIIb to Vb was

[1] Metrazol.

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