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Effect of Caffeine on Ergotamine Absorption from Rat Small Intestine

JOHN R. ANDERSON, GERTRUDE DREHSEN, and IAN H. PITMAN ˣ

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Abstract □ The effect of caffeine on the absorption of ergotamine from the rat small intestine was studied. The results of a series of experiments showed that caffeine significantly enhanced absorption of ergotamine from solutions of pH 5.0 when both substances were in solution and when an intact blood supply was either absent (*in vitro* everted sac experiments) or present (*in situ* experiments). Caffeine did not appear to influence the absorption rate of ergotamine in *in situ* experiments when the solution pH was 3.0. Isosorbide dinitrate, a vasodilator, enhanced ergotamine absorption when both substances were administered simultaneously into intestinal loops *in situ*. Isosorbide dinitrate probably exerts its effect by increasing blood flow to the intestine. The results are consistent with an hypothesis that the rate-determining step in ergotamine absorption is the transport of the drug from a lipid phase (GI membrane) into an aqueous phase (blood). Caffeine is thought to exert its rate-accelerating effect by increasing the water solubility of ergotamine neutral molecules.

Keyphrases □ Caffeine—effect on ergotamine absorption from rat small intestine, *in vitro* and *in vivo* studies □ Ergotamine—effect of caffeine on absorption from rat small intestine, *in vitro* and *in vivo* studies □ Absorption—ergotamine, effect of caffeine on absorption from rat small intestine, *in vitro* and *in vivo* studies □ Analgesics—ergotamine, effect of caffeine on absorption from rat small intestine, *in vitro* and *in vivo* studies

Ergotamine tartrate is widely used in the treatment of migraine (1, 2). When administered parenterally, it is effective in relieving migraine pain (3, 4), but oral administration often affords no relief (5, 6). Regardless of the route, ergotamine must be administered early in an attack to be effective (7). The frequent failure of oral ergotamine therapy appears to be related to its relatively low water solubility (1:500) and the fact that its absorption from the GI tract into the systemic circulation often is slow or impaired during a migraine attack (4–9).

Simultaneous oral administration of caffeine and ergotamine results in more effective therapy than ergotamine administered alone (10–12). Recent pharmacokinetic studies in humans indicated faster and more complete absorption of ergotamine after oral administration when it is combined with caffeine (13). Zoglio *et al.* (14) conducted *in vitro* experiments to elucidate the mechanism by which caffeine enhances ergotamine absorption; caffeine increased the solubility and dissolution rates of ergotamine in 0.1 *M* HCl and in 0.1 *M* phosphate buffer (pH 6.65) and was claimed to accelerate the partitioning of ergotamine from an aqueous (pH 6.65) to an organic (chloroform) phase. These observations led to the conclusion that the solubilizing effect of caffeine on ergotamine in water is the major factor in its enhancement of oral ergotamine migraine therapy.

The present study was undertaken to determine the effect, if any, that caffeine has on ergotamine absorption across the rat small intestine when both substances are dissolved.

EXPERIMENTAL

Ergotamine Assay—Ergotamine concentrations were calculated from the results of reversed-phase high-performance liquid chromatographic (HPLC) assays (15). The mobile phase was acetonitrile–1% sodium acetate/acetic acid buffer (55:45) at pH 6.5. The stationary phase was a 10/25 ODS column, and ergotamine was detected by fluorescence spectrometry (λ_{ex} = 325 nm; λ_{em} = 407 nm).

***In Vitro* Studies (Everted Sac Technique)**—Adult male Hooded Wistar rats, 240–280 g, were fasted in cages designed to prevent coprophagy for at least 12 hr before sacrifice. Water was allowed *ad libitum*. The rats were killed by a blow on the head. The small intestine was removed immediately, rinsed with Krebs–Henselite solution, sleeved onto a glass rod, and everted carefully. The first 15 cm, beginning with the pylorus, was discarded, and the next 8-cm segment was used in the experiments.

The segment was attached to a cannula at the proximal end, trimmed to 7 cm, and ligated at the distal end so that 6 cm of the everted intestine

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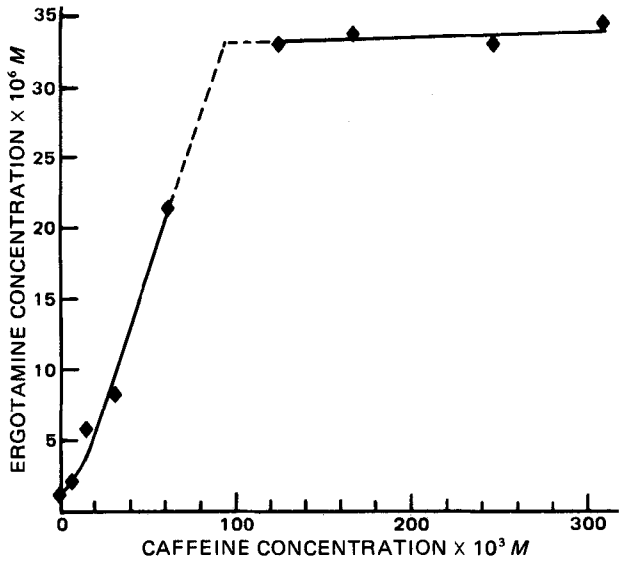


Figure 1—*Solubilizing effect of caffeine on ergotamine in pH 8.5 boric acid buffer. The shape of the diagram between* $[caffeine]_T = 60 \times 10^{-3}$ M *and* 120×10^{-3} M *was not determined. The initial solid phase was ergotamine tartrate.*

was exposed to the drug solution. The intestinal sac was maintained in a vertical position during the experiment. The organ bath was filled with 60.0 ml of buffered drug solution (mucosal solution), which was maintained at 37.0 ± 0.1° and continuously gassed with 95% O_2–5% CO_2. The inner (serosal) solution consisted of 0.6 ml of buffer.

The buffer for the mucosal and serosal solutions consisted of Krebs–Henseleit solution–0.1 *M* sodium acetate (9:1), buffered to pH 5.0 with acetic acid. Quinine sulfate (0.5 mg/ml) was included to act as the internal standard in the ergotamine assay. In all experiments, the mucosal solution was the same as the serosal solution plus 0.1 mg of ergotamine tartrate/ml. In permeation experiments involving the rate of ergotamine transfer across the membrane in the presence of caffeine, 10 mg of caffeine/ml was included in the mucosal and serosal solutions.

During the experiments, 10-μl samples were withdrawn from the serosal solution at indicated times and assayed for ergotamine by the HPLC method described previously (15). The concentration of ergotamine transferred across the membrane was expressed as a percentage of the ergotamine concentration in the mucosal solution. The transfer rates were determined from the slope of a plot of log (100 − percent of ergotamine absorbed) *versus* time.

***In Situ* Studies**—Five loops of intestine (12 cm in length) were prepared in each rat according to the method described previously (16). The total volume of test solution introduced into each loop was 1.0 ml.

Two types of experiments were conducted: "time zero" studies, in which the intestine was removed from the rat immediately after the test solutions were placed in the loops, and "absorption" studies, in which the intestine remained *in situ* within the abdominal cavity of the rat for 2.5 hr before being removed.

The absorption characteristics of ergotamine tartrate were investigated in the presence of two additives, caffeine and isosorbide dinitrate. The caffeine experiments were conducted at pH 3 (0.1 *M* tartaric acid–sodium hydroxide buffer), 5 (sodium acetate–acetic acid buffer), and 6 (0.1 *M* citric acid–sodium hydroxide buffer). In the isosorbide dinitrate experiments, all test and control solutions introduced into the loops were prepared in the pH 5.0 acetate buffer, which also contained 10% dimethyl sulfoxide to solubilize the isosorbide dinitrate. The ergotamine tartrate concentration was 0.05, 0.1, or 0.2 mg/ml, the caffeine concentration was either 10 or 20 mg/ml, and the isosorbide dinitrate concentration was 2 mg/ml.

In the caffeine experiments, two loops contained an ergotamine tartrate solution at a particular concentration while another two loops contained ergotamine tartrate at the same concentration plus a particular concentration of caffeine. The remaining loop in each rat contained the appropriate buffer solution (blank loop) and served as a reference for the assay. The contents of the five loops were randomized on the basis of rows from a 5 × 5 Latin square. Five rats were used at each ergotamine tartrate and caffeine combination to complete the Latin square.

In the isosorbide dinitrate experiments, two different methods of allocating the solutions to particular loops were used. One was on the basis of a 5 × 5 Latin square, with two loops containing ergotamine tartrate solution, two loops containing ergotamine tartrate plus isosorbide dinitrate solution, and the remaining loop containing the reference solution. The second method involved placing only one type of test solution into all five loops of two rats.

At the end of each experiment, the small intestine was removed from the rat, trimmed of its mesentery and blood vessels, and rinsed in 0.9% (w/v) NaCl solution. The length of each loop was measured and recorded. The individual loops were placed in separate homogenization flasks and manually chopped as finely as possible. Five milliliters of 1.0% (w/v) tartaric acid solution was added to each flask, and the contents were homogenized for 10 min.

The homogenates were transferred quantitatively with 10 ml of absolute ethanol to centrifuge tubes. These homogenates were centrifuged at 2000×*g* for 10 min, and the supernates were decanted into 50-ml volumetric flasks. Each precipitate was resuspended and washed with three 10-ml portions of 70% ethanol and centrifuged as before, and the washings were combined with their corresponding supernates. Five milliliters of a 200-μg/ml quinine sulfate (HPLC assay internal standard) solution in 70% ethanol was added to each flask. The combined solutions were filtered through 0.80-μm membrane filters to remove solid material before they were assayed (15). Ergotamine that could not be recovered by this method was considered to have been absorbed.

A preliminary experiment was conducted to ensure that the rat intestinal secretions did not significantly alter the pH values of the test solutions during absorption. Five milliliters of the pH 5.0 acetate buffer was introduced into the rat small intestine. After 2.5 hr, the intestine was removed from the abdominal cavity, and the fluid in the lumen of the intestine was collected. The intestinal fluid was filtered through a 0.22-μm membrane filter, and the filtrate pH was measured.

Solubility Studies—Excess quantities of ergotamine tartrate (50 mg) were placed in 25-ml screw-capped vials together with varying, but accurately weighed, amounts of caffeine (0–480 mg) and 10 ml of pH 8.5 borate buffer (4.5 ml of 0.2 *M* boric acid and 5.5 ml of 0.05 *M* borax). The vials were placed in a constant-temperature water bath and shaken for 48 hr at 25°. Preliminary studies were conducted to determine the time required for the solutions to reach equilibrium.

The total amount of ergotamine in each equilibrated solution was determined by filtering the vial contents through a 0.22-μm membrane filter and analyzing for ergotamine concentration using the previously described HPLC method (15).

RESULTS

Solubility Studies—The phase solubility diagram, obtained by saturation of caffeine solutions in pH 8.5 boric acid buffers with ergotamine tartrate, is shown in Fig. 1. The addition of 0.124 *M* caffeine increased ergotamine solubility at pH 8.5 from 1.25×10^{-6} to 3.29×10^{-5} *M*. Additional caffeine did not significantly alter ergotamine solubility.

***In Vitro* Studies (Everted Sac Technique)**—The absorption of ergotamine across the rat small intestine was initially studied using a modification of a reported everted sac technique (17). The volume of liquid and the concentration of quinine sulfate (internal standard) on both the serosal and mucosal sides of the everted intestine did not change appreciably during the experiment. Therefore, it was concluded that no net transfer of water or of quinine sulfate took place.

The transfer rates of ergotamine across the membrane were calculated from the slopes of plots of log (100 − percent of ergotamine absorbed) *versus* time (Fig. 2). A *t* test indicated that caffeine significantly increased the transfer rate of ergotamine from solution across the isolated rat intestine ($p < 0.001$).

***In Situ* Studies Involving Caffeine and Ergotamine**—The *in situ* technique used to measure the absorption[1] of ergotamine from the rat small intestine was based on a method developed in these laboratories by Admans *et al.* (16). It was a modification of the technique of Levine and Pelikan (18), which allows absorption of drugs from single or multiple intestinal loops to be measured.

Ergotamine solutions were introduced into four intestinal loops in an anesthetized rat, and a blank solution was introduced into a fifth loop. After 2.5 hr, the loops and their contents were homogenized separately and assayed for ergotamine. The amount of ergotamine absorbed was calculated from the difference between the amount administered and the amount remaining and was expressed as the percent absorbed.

A minor complication in calculating the amount of ergotamine remaining arose because HPLC chromatograms of ergotamine tartrate solutions that had been in the rat gut for 2.5 hr contained two more peaks than those observed for an ergotamine solution. The additional peaks

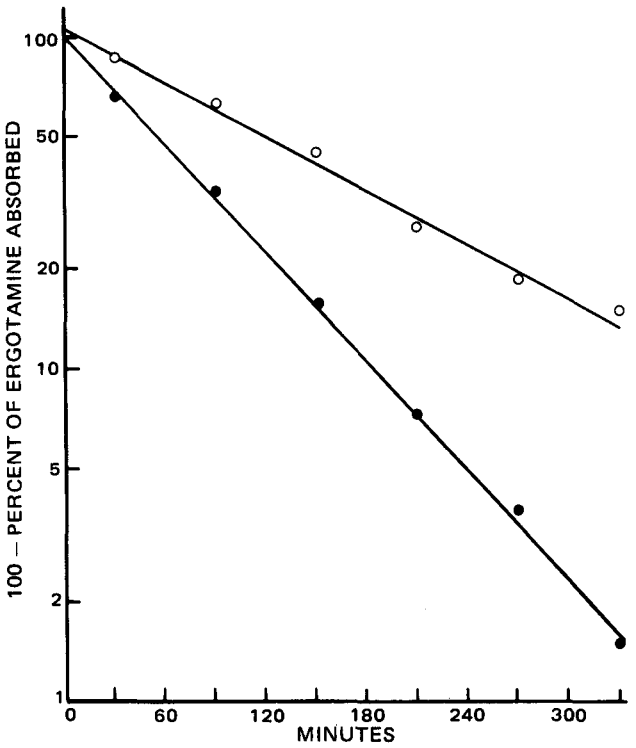


Figure 2—*Semilogarithmic plot against time of (100 − percent of ergotamine absorbed) in everted rat gut experiments. Key: ○, 0.1 mg of ergotamine tartrate/ml, slope = -6.23×10^{-3}/min; and ●, 0.1 mg of ergotamine tartrate/ml plus 10 mg of caffeine/ml, slope = -1.26×10^{-2}/min.*

had retention times of 6.5 and 7.5 min, whereas the ergotamine peak had a retention time of 9.0 min. Typical chromatograms that were measured from experiments conducted at pH 3 and 5 are shown in Fig. 3. In the experiments at pH 3.0, the metabolites or degradation product peaks were barely apparent. At pH 5.0, the metabolism was greater than at pH 3.0 but still relatively slight. When experiments at pH 6.0 were attempted, little ergotamine remained in the loops after 2.5 hr and the metabolites were significant, thereby making quantitation of absorption difficult.

Ergotaminine, an isomer of ergotamine that results from epimerization at C-8 (19), gave a peak with a retention time of 6.5 min. Thus, it was concluded that ergotaminine was one degradation product. Because fluorescence of ergotaminine is 2.5 times greater than that of ergotamine (20), it was estimated that the ratio of the amount of ergotaminine to the original amount of ergotamine after 2.5 hr at pH 5.0 was 0.03:1. Thus, conversion by metabolism of ergotamine to ergotaminine is unlikely to affect the results significantly.

The extraneous peak with a retention time of 7.5 min was twice as intense as that of ergotaminine. The nature of the compound causing this peak is uncertain, but it is likely to be *aci*-ergotamine or *aci*-ergotaminine, which is formed by the inversion of ergotamine or ergotaminine at the 2′-position (19). These compounds are more polar than ergotamine (21) and are expected to be eluted earlier with the mobile phase employed. Although the fluorescence intensity of the *aci* compounds is not known, it is unlikely that they would fluoresce much less strongly than ergotamine.

In the absence of more precise information, it was assumed that the fluorescence of the metabolites was similar to that of ergotamine. Hence, ergotamine concentrations that remained were derived by summing the areas under the peaks for ergotamine and its metabolites. This procedure should introduce a maximum error of ±10% into the results[1].

***In Situ* Studies**—*pH Value of Intestinal Contents*—The pH of the intestinal contents 2.5 hr after introduction of the pH 5.0 acetate buffer was 5.3. The pKa value of ergotamine is 6.4 (22). Therefore, at least 90% of the ergotamine existed in the ionized form and remained in solution throughout the study. It also was found that a 0.2-mg/ml solution of ergotamine tartrate could be obtained at pH 5.3. Since the maximum concentration of ergotamine tartrate introduced into the intestinal loops was 0.2 mg/ml, all of the ergotamine in the intestinal loops, either by itself or in the presence of caffeine, probably remained in solution throughout the absorption experiments conducted at pH 5 or below.

[1] A reviewer pointed out that the above experiments do not distinguish between absorption and loss of ergotamine due to metabolism if the metabolites are themselves absorbed. This point is accepted but, in the absence of other information, the good correlations between the effect of caffeine on ergotamine absorption in *in vitro* and *in situ* experiments and the low probability that caffeine appreciably influences ergotamine metabolism are accepted as points that support the discussion in the manuscript.

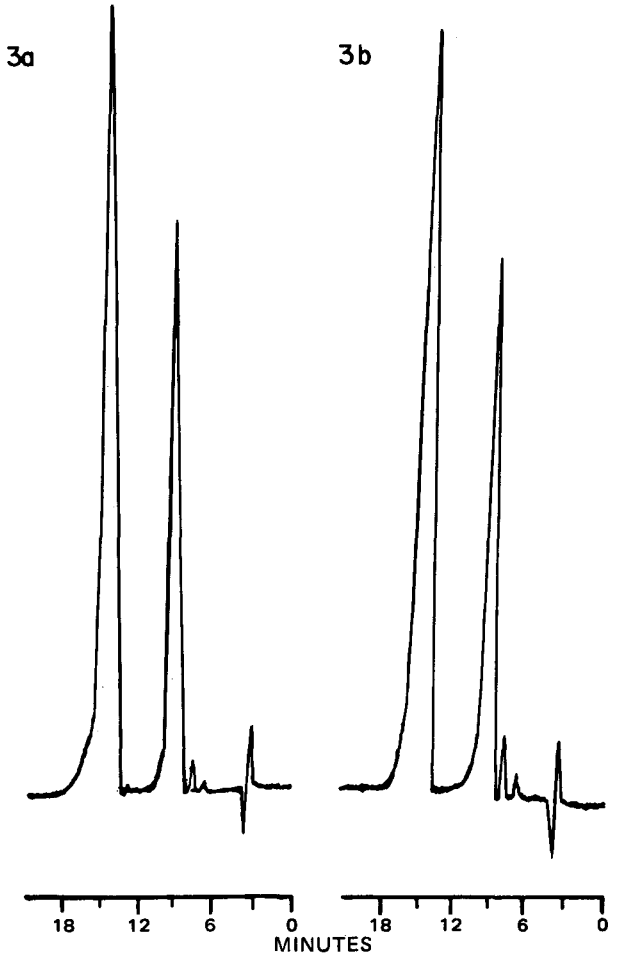


Figure 3—*Chromatograms of extracts from* in situ *rat intestinal homogenates. Experiments were conducted at pH 3.0 (a) and 5.0 (b). The peaks eluted after 9 and 15 min were ergotamine and quinine, respectively.*

Significance of Blank Loop and Effect of Loop Length—Ergotamine could disappear from a loop by either absorption into the blood or lymphatic system or by leakage into an adjoining loop. Ergotamine also could possibly be reabsorbed from the blood into the lumen of a loop. However, no ergotamine was detected in the loop containing the blank solution. Hence, it was concluded that ergotamine that had disappeared from a loop was absorbed.

An analysis of variance of the results indicated that the variation in loop length that occurred with this technique had no significant effect on the percentage of the administered amount of ergotamine absorbed from the loops ($p > 0.1$).

Effect of Caffeine—In the experiments designed to test the reliability of the recovery process (time zero studies), loops containing either 0.2 mg of ergotamine tartrate/ml alone or 0.2 mg of ergotamine tartrate/ml plus 20 mg of caffeine/ml were removed and assayed immediately after introduction of the test solutions into the loops. At pH 5.0, the mean recovery of ergotamine from 10 loops (five rats) was 97 ± 3%. The mean recovery of ergotamine in the presence of caffeine from 10 loops (five rats) was 98 ± 3%. At pH 3.0, the recoveries were 95 ± 6% for ergotamine tartrate solution (four loops) and 96 ± 1% for ergotamine tartrate and caffeine solution (four loops).

Analysis of variance conducted on these results indicated that caffeine had no significant effect on the recovery of ergotamine from loops at pH 3.0 ($p > 0.1$) or 5.0 ($p > 0.1$) and that the loop position (*i.e.*, distance from the pylorus) had no significant effect on the amount of ergotamine re-

Table I—Extent of Ergotamine Absorption from *In Situ* Rat Small Intestinal Loops after 2.5 hr in the Presence and Absence of Caffeine

pH	Ergotamine Tartrate, mg/ml	Caffeine, mg/ml	Absorption of Ergotamine[a], % Without Caffeine	Absorption of Ergotamine[a], % With Caffeine
5.0	0.2	20	21 (6)	61 (6)
	0.2	10	24 (9)	49 (5)
	0.1	10	25 (9)	53 (6)
3.0	0.2	10	47 (14)	47 (12)

[a] Each value is the mean of 10 loops from five rats. Standard deviation is in parentheses.

covered ($p > 0.1$). Because of the near complete recovery of ergotamine at time zero, no correction to the results obtained at 2.5 hr was necessary.

The results of the absorption studies are shown in Table I. Analysis of variance indicated that in each of three sets of experiments at pH 5.0, the presence of caffeine in the loops caused a highly significant increase in the extent of ergotamine absorption ($p < 0.001$). A t test conducted on the results of the 0.2-mg/ml ergotamine tartrate experiments indicated that 20 mg of caffeine/ml had a significantly greater effect on ergotamine absorption than did 10 mg of caffeine/ml ($p < 0.001$). No significant difference was found in the percentage of ergotamine absorbed from loops containing ergotamine tartrate alone at 0.1 or 0.2 mg/ml ($p > 0.1$).

At pH 3.0, analysis of variance indicated that the presence of 20 mg of caffeine/ml in the loops had no significant effect on the extent of absorption of ergotamine ($p > 0.05$).

The position of the loop relative to the pylorus also had no significant effect on the amount of ergotamine absorbed at pH 3.0 or 5.0 ($p > 0.1$).

Effect of Isosorbide Dinitrate—The efficiency of the recovery process was demonstrated in the time zero experiments. The mean recovery of ergotamine from 10 loops (five rats) containing 0.2 mg of ergotamine tartrate/ml was 82 ± 5%; in the presence of 2 mg of isosorbide dinitrate/ml, it was 83 ± 5%. Analysis of variance demonstrated that neither the presence of isosorbide dinitrate nor loop position had a significant effect on ergotamine recovery ($p > 0.1$).

In the caffeine experiments, the recovery of ergotamine from loops containing 0.2 mg of ergotamine tartrate/ml alone was 97 ± 3%. The major difference between the caffeine and isosorbide dinitrate recovery experiments was the inclusion, for solubility, of 10% dimethyl sulfoxide in the pH 5.0 acetate buffer in the latter experiments. Comparison of the results using a t test showed that dimethyl sulfoxide significantly affected ergotamine recovery ($p < 0.001$), but the reason for this effect is not known. The results are listed in Table II.

The ergotamine absorption figures are corrected by the factor "100/percentage of ergotamine recovered in time zero studies." This correction was necessary since the time zero studies indicated that extraction did not produce complete recovery of the ergotamine remaining in the loop. Analysis of variance indicated that isosorbide dinitrate had a significant effect on ergotamine absorption at the 5% confidence level ($0.05 > p > 0.01$) when ergotamine alone and when ergotamine plus isosorbide dinitrate were administered to the same rats. Loop position had no significant effect on absorption ($p > 0.1$).

In the second series of isosorbide dinitrate experiments, all five loops of a rat contained only one test solution. The corrected figures for the mean percentage absorption of ergotamine from loops containing ergotamine tartrate alone and from loops containing ergotamine tartrate plus isosorbide dinitrate were 19 ± 12 and 39 ± 8%, respectively. A t test indicated that ergotamine absorption in rats in which all loops contained ergotamine tartrate plus isosorbide dinitrate was significantly greater than in rats in which all loops contained only ergotamine tartrate ($p < 0.001$).

The effect of isosorbide dinitrate in loops adjacent to those containing ergotamine tartrate alone was tested by comparing ergotamine absorption from the rats in the first series of experiments, where the adjacent loops contained isosorbide dinitrate, with the rats in the second series of experiments where all loops contained ergotamine tartrate only. The absorption in these two cases was 34 ± 7 and 19 ± 12%, respectively. A t test indicated that isosorbide dinitrate in intestinal loops adjacent to the loops under study significantly increased ergotamine absorption, compared to similar loops in rats in which no isosorbide dinitrate was present ($0.01 > p > 0.002$).

DISCUSSION

It was established previously that caffeine and ergotamine form one or more molecular complexes in water, but no attempt was made to calculate equilibrium constants for these reactions (14, 23).

Figure 1 shows the phase solubility diagram of ergotamine neutral molecule (E) in solutions of neutral caffeine (C). Measurements were made at pH 8 5. Caffeine was within its solubility when its concentrations were between 0 and 7×10^{-2} M (24). Solutions to which 12×10^{-2} M or more caffeine had been added were saturated with respect to caffeine. These results are consistent with ergotamine and caffeine forming 1:1 (EC) and 1:2 (EC_2) complexes (Schemes I and II):

$$E + C \overset{K_1}{\rightleftharpoons} EC$$

Scheme I

$$EC + C \overset{K_2}{\rightleftharpoons} EC_2$$

Scheme II

where:

$$K_1 = \frac{[EC]}{[E][C]} \qquad \text{(Eq. 1)}$$

$$K_2 = \frac{[EC_2]}{[EC][C]} \qquad \text{(Eq. 2)}$$

According to this model, the total amount of ergotamine in solution under any set of conditions, $[E]_T$, would be:

$$[E]_T = [E] + [EC] + [EC_2] \qquad \text{(Eq. 3)}$$

and the total amount of caffeine in solution, $[C]_T$, would be:

$$[C]_T = [C] + [EC] + 2[EC_2] \qquad \text{(Eq. 4)}$$

Because $[E]_T$ only varied between 1 and 34×10^{-6} M when the added caffeine concentration was varied between 0 and 6×10^{-2} M, it could be assumed that $[C]_T \equiv [C]$ in all experiments performed.

On the ascending portion of the curve (*i.e.*, where the solutions were saturated with respect to ergotamine but not with respect to caffeine), [E] is the value of $[E]_T$ when [C] = 0.

Substitution of Eqs. 1 and 2 in Eq. 3 and rearrangement yield:

$$\frac{[E]_T - [E]}{[E][C]} = K_1 + K_1K_2[C] \qquad \text{(Eq. 5)}$$

Hence, if the model is compatible with the data, a plot of the left side of Eq. 5 against [C] should be linear. The value of the intercept of this line when [C] = 0 would be K_1, and the slope to intercept ratio would be K_2.

The results in Fig. 4 show that this plot is essentially linear. A least-

Table II—Absorption of Ergotamine from the Rat Small Intestine in the Presence and Absence of Isosorbide Dinitrate at pH 5.0

Test Solution	Mean Percentage of Ergotamine Extracted from Loops after 2.5 hr	Mean Corrected Percentage of Ergotamine Remaining in Loops after 2.5 hr	Mean Percent Absorption of Ergotamine after 2.5 hr
Ergotamine tartrate, 0.2 mg/ml	54.0 ± 6.0[a]	65.9 ± 7.3[a]	34.1[a]
	66.4 ± 9.8[b]	81.0 ± 12.0[b]	19.0[b]
Ergotamine tartrate, 0.2 mg/ml, plus isosorbide dinitrate, 2 mg/ml	48.2 ± 6.2[a]	57.9 ± 7.5[a]	42.1[a]
	50.6 ± 9.8[b]	61.0 ± 8.0[b]	39.0[b]

[a] Both solutions were administered to each rat. [b] Either ergotamine tartrate alone or ergotamine tartrate plus isosorbide dinitrate was administered to each rat.

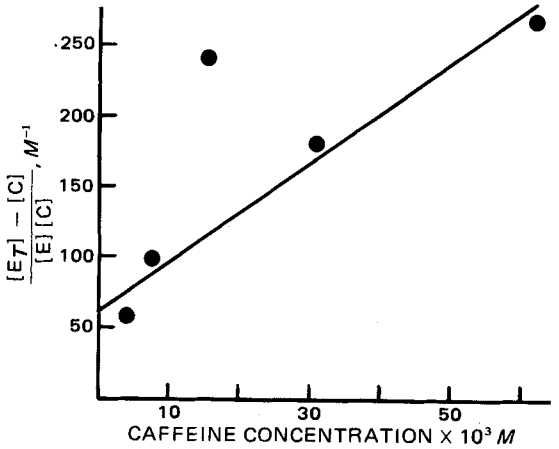


Figure 4—*Plot of $([E]_T - [E])/[E][C]$ against [C] for complexation of caffeine and ergotamine at pH 8.5.*

squares linear regression ($r^2 = 0.974$) of the results yielded values for K_1 and K_2 of 62 and 55 M^{-1}, respectively.

One test of the validity of this model comes from consideration of the results obtained in solutions saturated with caffeine (*i.e.*, when the concentration of added caffeine was greater than 12×10^{-2} *M*). Under these conditions, a rearranged form of Eq. 5 suggests:

$$[E]_T = [E](1 + K_1[C]_S + K_1K_2[C]_S^2) \qquad \text{(Eq. 6)}$$

where $[C]_S$ is the caffeine solubility. It can be seen from Fig. 1 that, as required by Eq. 6, the value of $[E]_T$ is essentially independent of the concentration of added caffeine when the solubility of caffeine is exceeded. Solution of Eq. 6 for the solubility of caffeine yields a value of 8–9 $\times 10^{-2}$ *M*. Previous studies (24) showed that caffeine has a solubility of 1.8×10^{-1} *M* in water but that this value was depressed by increasing the ionic strength (*e.g.*, 4.5×10^{-2} *M* at pH > 8.5 when the ionic strength was adjusted to 3.0 *M* with sodium chloride). Hence, a value for caffeine solubility between 8 and 9×10^{-2} *M* in a solution containing buffer salts and saturated with respect to ergotamine appears to be reasonable.

A similar analysis was made of the phase solubility diagram of ergotamine in caffeine solutions at pH 2.0 (23). Under these conditions, ergotamine is essentially completely protonated (EH^+), and caffeine is essentially present as a neutral molecule. The 1:1 (ECH^+) and 1:2 (EC_2H^+) complexes probably also would be essentially completely protonated (Schemes III and IV). Hence, the relevant reactions would be:

$$EH^+ + C \overset{K_3}{\rightleftharpoons} ECH^+$$

Scheme III

$$ECH^+ + C \overset{K_4}{\rightleftharpoons} EC_2H^+$$

Scheme IV

By similar reasoning to that outlined for reactions at pH 8.5, this model requires that a plot of $([E]_T - [EH^+])/([EH^+][C])$ against [C] should be linear with the intercept when [C] = 0 is equal to K_3 and the slope to intercept value is equal to K_4. A linear regression analysis of the results in Fig. 5 (which were computed from data in Ref. 23) yielded values of K_3 and K_4 of 204 and 44 M^{-1}, respectively. The standard deviation of the line was 0.98.

Consideration of the values of K_1, K_2, K_3, and K_4 gives some insight into the nature of the complexation reaction. Hence, the strongest complexation reaction involves caffeine and ergotamine cation (K_3 = 204 M^{-1}). The positive charge in the complex that is formed (ECH^+) apparently must be largely neutralized or buried since the complexation of caffeine with ECH^+ (K_4 = 44 M^{-1}) is a similar reaction to that of caffeine with the ergotamine neutral molecule (K_1 = 62 M^{-1}) or the neutral caffeine–ergotamine complex (K_2 = 55 M^{-1}).

***In Vitro* Absorption of Ergotamine**—The results in Fig. 2 show clearly that caffeine (10 mg/ml) significantly increased the rate at which ergotamine (initially 0.1 mg/ml) passed from the mucosal to the serosal side of the rat intestine when the pH of the contents of the former side was 5.0.

One important feature is that ergotamine was in solution on the mucosal side of the membrane. Hence, caffeine could not exert its accelerating effect on ergotamine absorption by increasing the dissolution rate of solid ergotamine. Caffeine's role in this latter process was suggested as its major role in enhancing ergotamine absorption following its administration as a solid (14).

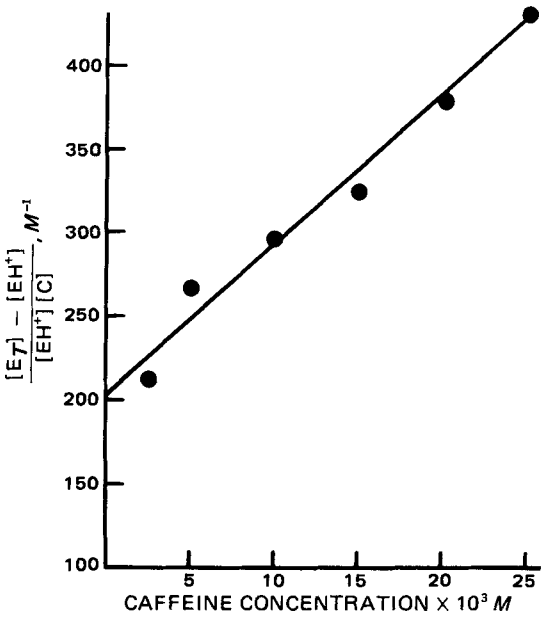


Figure 5—*Plot of $([E]_T - [EH^+])/[EH^+][C]$ against [C] for complexation of caffeine and ergotamine cation at pH 2.0.*

Another feature is that no blood supply was available to the intestine. Consequently, caffeine could not exert its effect by changing the blood flow rate. These results will be discussed in conjunction with the results of the *in situ* studies.

***In Situ* Absorption of Ergotamine**—The *in situ* experiments differed from the *in vitro* experiments in that an intact blood supply was present. However, as in the former experiments, ergotamine was in solution in the intestinal lumen, so the dissolution rate could not be a rate-determining factor in absorption. The results of experiments at pH 3 and 5 in both the absence and presence of caffeine are presented in Table I.

One point of interest concerns the effect, or apparent lack of effect, that varying the pH from 3 to 5 had on the absorption of the weakly basic ergotamine (pKa of conjugate acid = 6.4). Hence, although the absorption rate of ergotamine was similar at pH 3 and 5, the percent composition of the ergotamine neutral molecule in solution varied approximately 100-fold, from 0.04% at pH 3 to 3.8% at pH 5. Throughout the same pH range, the composition with respect to ergotamine cation varied from 99.96 to 96.2%.

The results in Table I also show that, at pH 3.0, the absorption rate of ergotamine (initial concentration of ergotamine tartrate 0.2 mg/ml) was the same in either the absence or the presence of caffeine (10 mg/ml). The solutions that did not contain caffeine contained 99.96% ergotamine cation and 0.04% ergotamine neutral molecule. The composition of the solutions with respect to ergotamine species are shown in Table III. Values of K_1, K_2, K_3, and K_4 for the reactions shown in Schemes I–IV, respectively, and a K_a value for ergotamine of 3.98×10^{-7} *M* were used to compute these results. Hence, while the concentration of ergotamine cation decreased from 99.96% in the absence of caffeine to 2.8%, the total concentration of ergotamine cation plus protonated complexes remained essentially constant.

The situation at pH 5.0 was quite different, because caffeine (10 or 20 mg/ml) significantly increased the absorption rate of ergotamine. The extent to which caffeine increased the absorption rate in the *in situ* experiments was similar to that in the *in vitro* experiments.

Table III—Composition [a], as a Percentage of Total Ergotamine Species, of Solutions Containing Ergotamine (0.2 mg/ml) and Caffeine (10 mg/ml) at pH 3.0 and 5.0

	Concentration, %					
pH	EH^+	ECH^+	EC_2H^+	E	EC	EC_2
3.0	2.8	29.7	67.6	1.1×10^{-3}	3.6×10^{-3}	1.0×10^{-2}
5.0	2.8	29.2	66.6	1.1×10^{-1}	3.5×10^{-1}	1.0

[a] Calculated as described in the text.

These results are consistent with a model for GI absorption of basic drugs described earlier (25). The appropriate model (Model A in Ref. 25) assumes that neutral molecules, but not ions, rapidly pass from the aqueous environment of the lumen into the lipoidal GI membrane and that equilibrium between these two phases is established rapidly. The slow step in overall absorption is the transport of neutral molecules from the lipoidal membrane into the aqueous blood compartment. The model for ergotamine absorption is shown schematically in Scheme V. According to this model, the rate of drug appearance in the blood is proportional to the concentration of neutral drug molecules in the membrane. Hence:

$$\text{absorption rate} = k_E[E]_m V_m + k_{EC}[EC]_m V_m + k_{EC_2}[EC_2]_m V_m \quad \text{(Eq. 7)}$$

where the terms in brackets with subscript m, $[\ ]_m$, refer to the concentration of the various species in the membrane and V_m is the volume of the membrane. By definition:

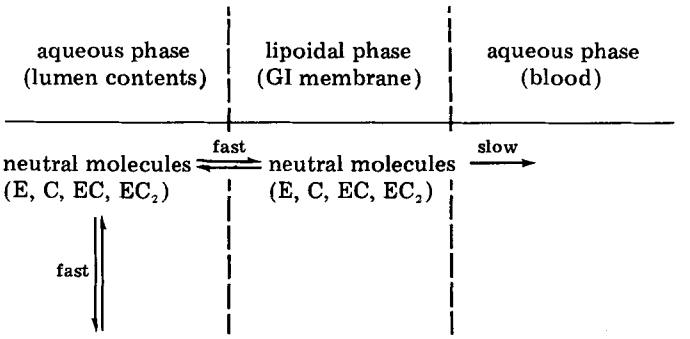
$$P_E = \frac{[E]_m}{[E]_w} \quad \text{(Eq. 8)}$$

$$P_{EC} = \frac{[EC]_m}{[EC]_w} \quad \text{(Eq. 9)}$$

$$P_{EC_2} = \frac{[EC_2]_m}{[EC_2]_w} \quad \text{(Eq. 10)}$$

where the P symbols are the intrinsic partition coefficients for the three ergotamine species between the aqueous contents of the lumen and the membrane and the terms in brackets with subscript w, $[\ ]_w$, refer to the concentrations of the various species in water.

Substitution of Eqs. 8–10 in Eq. 7 and expression of the various concentration terms as fractions of the total amount of ergotamine in the lumen contents and in the membrane E_T yield:



Scheme V

absorption rate did not change as $[H^+]$ increased from 10^{-5} to 10^{-3} M if the value of $P_E \gg (V_w/V_m)\ 2.5 \times 10^3$. This seems reasonable since P_E is expected to be very large. The Merck Index (26) states that ergotamine is "freely soluble in chloroform" and "only slightly soluble in water."

The approximate doubling of the rate of ergotamine absorption in the presence of caffeine (5.15×10^{-2} M) when the pH was increased from 3 to 5 needs to be explained. The model would be consistent with this result if, in the presence of caffeine, the term in the denominator that contained $[H^+]$ and the term containing P_E were of comparable value at pH 3. But if the term containing P_E was much greater than the term containing $[H^+]$ at pH 5, although still being slightly less than the value of P_E, knowledge of the values of the equilibrium constants K_1, K_2, K_3, and K_4 indicate that the term containing $[H^+]$ would be greater in the presence of 5.15×10^{-2} M caffeine than in its absence by a factor of 2.7. The value of the term containing P_E is not known precisely, but it is reasonable to expect that it would be less than P_E. This situation would arise if the values of the partition coefficients P_{EC} and P_{EC_2} were smaller than P_E. Although the values of these terms are not known, the phase solubility study (Fig. 1) reveals that the complexes EC and EC_2 have significantly higher water solubility than ergotamine. Hence, it is not unreasonable to assume that they will have a smaller tendency than ergotamine to partition into a lipoidal phase.

$$\text{rate of absorption} = \frac{(k_E P_E K_a + k_{EC} P_{EC} K_1 K_a [C]_w + k_{EC_2} P_{EC_2} K_1 K_2 K_a [C]_w^2) E_T}{\frac{V_w}{V_m}[K_a + 1 + K_3[C]_w + K_3 K_4 [C]_w^2 [H^+] + K_1 K_a [C]_w + K_1 K_2 K_a [C]_w^2] + K_a (P_E + P_{EC} K_1 [C]_w + P_{EC_2} K_1 K_2 [C]_w^2)} \quad \text{(Eq. 11)}$$

where V_w is the volume of the aqueous lumen contents and $[C]_w$ is the concentration of caffeine in the lumen contents. If transport of a species, i, from the lipoidal membrane across an aqueous barrier and into the blood is the rate-determining process, then, from Fick's first law:

$$k_i = \frac{D_i A_i P_i}{h_i} \quad \text{(Eq. 12)}$$

where D_i is the diffusion coefficient of the i species in an aqueous barrier of surface area A_i and thickness h_i, and P_i is the partition coefficient of the species i from the lipoidal membrane into the aqueous phase. This model assumes transport into a sink. If the following additional assumptions are made:

1. The values of the diffusion coefficients for the species E, EC, and EC_2 are equal and have the value D
2. The partition coefficients of the species from the lipoidal membrane into blood are the inverse of the P values in Eq. 11, which were for partitioning from the aqueous lumen contents into the membrane

then substitution of the terms defined in Eq. 12 into Eq. 11 and rearrangement lead to:

$$\text{rate of absorption} = \frac{\frac{DA}{h} E_T}{\frac{V_w}{V_m} + \frac{V_w}{V_m}\frac{[H^+]}{K_a}\left\{\frac{1 + K_3[C]_w + K_3 K_4 [C]_w^2}{1 + K_1[C]_w + K_1 K_2 [C]_w^2}\right\} + P_E\left\{\frac{1 + \frac{P_{EC}}{P_E} K_1 [C]_w + \frac{P_{EC_2}}{P_E} K_1 K_2 [C]_w^2}{1 + K_1[C]_w + K_1 K_2 [C]_w^2}\right\}} \quad \text{(Eq. 13)}$$

where A and h are the surface area and thickness of the barrier.

When caffeine is absent, Eq. 13 reduces to:

$$\text{rate of absorption} = \frac{\frac{DA}{h} E_T}{\frac{V_w}{V_m} + \frac{V_w}{V_m}\frac{[H^+]}{K_a} + P_E} \quad \text{(Eq. 14)}$$

Hence, the model would be consistent with the data that showed that the

This model leads to the conclusion that the water-solubilizing effect of caffeine on ergotamine neutral molecules is a major factor in its accelerating effect on ergotamine absorption.

Zoglio and coworkers (14, 27, 28), in discussing the rate-accelerating effect of caffeine on the partitioning of ergotamine and related alkaloids from water to chloroform at pH 6.5, but not at pH 1.0, proposed that it did so by "preventing precipitation" of ergotamine prior to its absorption. In the present study, precipitation was not possible because solutions were not saturated with respect to ergotamine. However, the two theories are consistent in that both credit caffeine's role to the increased water solubility that it confers on the ergotamine neutral molecule.

Effect of Isosorbide Dinitrate on Ergotamine Absorption—Isosorbide dinitrate significantly increased the rate of ergotamine absorption. However, the nature of its effect was quite different than that of caffeine. For example, the presence of caffeine in intestinal loops adjacent to loops in the same rat that only contained ergotamine did not significantly increase the rate of ergotamine absorption. On the other hand, the presence of isosorbide dinitrate in any intestinal loop increased the rate of ergotamine absorption in other loops in the rat that did not contain isosorbide dinitrate.

Since isosorbide dinitrate is a vasodilator (29), it probably increases the rate of ergotamine absorption by increasing the rate of intestinal blood flow. This conclusion is consistent with the earlier postulate that the rate-determining step in ergotamine absorption is its rate of passage from the membrane into the blood rather than its passage into or through the membrane.

Caffeine is also a vasodilator (30); at a concentration of 2 mg/ml, it increased intestinal blood flow by 166% in anesthetized rats and increased the rate of absorption of tritiated water, urea, and antipyrine at pH 8.0 and of salicylic acid at pH 6 (31). Hence, while part of the effect that caffeine has on increasing the absorption of ergotamine at pH 5.0 may be due to its effect on the blood flow rate, this effect is thought to be minor because it does not affect absorption rates at pH 3, it does not affect absorption rates at pH 5 from adjacent loops that do not contain caffeine, and it exerts an accelerating effect on ergotamine absorption in *in vitro* experiments where no blood supply is present.

CONCLUSION

1. Caffeine increases the rate of ergotamine absorption from the rat small intestine when the pH of the intestinal contents is 5.0 but not when it is 3.0. This effect is independent of the effect that caffeine exerts on the dissolution rate of ergotamine because, in the experiments performed, ergotamine was administered as a stable solution.

2. Although caffeine increases intestinal blood flow, this property only plays a minor role in its absorption-accelerating effects at pH 5.0. Vasodilation and increased blood flow are likely to explain the rate-accelerating effect of isosorbide dinitrate on ergotamine absorption.

3. The rate-determining step in the absorption of ergotamine appears to be transport from the intestinal membrane into the blood.

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Effect of Poly-2-vinylpyridine-*N*-oxide and Sucrose on Silicate-Induced Hemolysis of Erythrocytes

DENNIS W. OSCARSON *, GEORGE E. VAN SCOYOC ‡x, and JAMES L. AHLRICHS ‡

Received May 9, 1980, from the **Saskatchewan Institute of Pedology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0,* and the ‡*Department of Agronomy, Purdue University, West Lafayette, IN 47907.* Accepted for publication November 19, 1980.

Abstract □ The biological activity of montmorillonite, palygorskite, kaolinite, chrysotile, and silica was examined using *in vitro* hemolysis of erythrocytes. The hemolytic potency was in the order montmorillonite > silica > palygorskite > chrysotile > kaolinite. The polymer poly-2-vinylpyridine-*N*-oxide inhibited hemolysis caused by montmorillonite, palygorskite, kaolinite, and silica, but it was less effective with chrysotile. The extent of polymer binding to the silicates and red blood cells was measured by UV spectroscopy. When sucrose was substituted for the saline solution as the incubating medium, hemolysis was eliminated in all systems except chrysotile–erythrocyte, where it was enhanced. The results indicate that both hydrogen bonding and ionic interactions between silicate surfaces and the erythrocyte membrane are important in the hemolytic process.

Keyphrases □ Silicates—effect of poly-2-vinylpyridine-*N*-oxide on silicate-induced hemolysis □ Hemolysis—effect of poly-2-vinylpyridine-*N*-oxide, silicates □ Suspending agents—effect of poly-2-vinylpyridine-*N*-oxide on silicate-induced hemolysis

Inhaled silicate particles cause various pathological disorders (1). In addition to pulmonary tissue damage, the particles can potentially damage other body tissues because silicates such as montmorillonite, kaolinite, and palygorskite are used in many pharmaceuticals and cosmetics (primarily as fillers, stabilizers, and suspending

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