Complexes of Ergot Alkaloids and Derivatives I: The Interaction of Caffeine with Ergotamine Tartrate in Aqueous Solution

M. A. ZOGLIO, H. V. MAULDING, JR., and J. J. WINDHEUSER

Abstract □ The interaction between ergotamine tartrate and caffeine was studied in distilled water, at pH 1 and at pH 6.65 with marked changes in solubility of the alkaloid being observed with increased caffeine concentration. Caffeine enhanced the dissolution rate of ergotamine tartrate by a factor of three at gastric pH. The partitioning rate of ergotamine tartrate from an aqueous to an organic phase was influenced by caffeine.

Keyphrases □ Ergot alkaloids, derivatives—complexes □ Caffeine-ergotamine tartrate—interaction □ Dissolution rate, ergotamine tartrate—caffeine effect □ pH effect—ergotamine tartrate solubility □ Partition rates, ergotamine tartrate—caffeine effect

The use of caffeine and ergotamine tartrate in combination has long been known to be effective in the treatment of migraine headache (1, 2). The effectiveness of this combination may be due, at least in part, to complex formation leading to enhanced enteral absorption of ergotamine.

Studies leading to this conclusion began when it was observed that addition of caffeine increased the aqueous solubility of ergotamine tartrate. In order to define this enhanced solubility, the effect of caffeine on the apparent solubility of ergotamine tartrate in aqueous media at several caffeine concentrations was studied. The contribution of this solubility effect to dissolution rate at gastric pH was measured. Partitioning rate studies (buffered aqueous to organic phase) were also included to help formulate an *in vitro* explanation for the efficacy of the ergotamine tartrate-caffeine combination.

EXPERIMENTAL

Solubility Studies—Watertight screw-capped vials (18-ml. capacity) containing exactly 10 ml. of solvent, 200 mg. of ergotamine tartrate, and varying amounts of caffeine were clamped onto the edge of metal disks 15.24-cm. (6-in.) diameter mounted on a motor-driven shaft and rotated vertically at 6 r.p.m. in a constant-temperature bath at 30° (±0.1°). After exactly 24 hr., samples were withdrawn using pipets with filters attached and analyzed for ergotamine tartrate by the Van Urk method (3).

Dissolution Rates—A 60-r.p.m. stirrer with a 2.54-cm. (1-in.) propeller blade placed 4 cm. from the bottom of a 800-ml. beaker containing 500 ml. of 0.1 *N* hydrochloric acid was used in determining these rates. The temperature of the dissolution rate media was kept at 30° (±0.1°) by immersing the beaker in a constant-temperature bath. Samples were withdrawn for analysis using pipets with filters attached and analyzed for ergotamine tartrate content by the Van Urk method (3).

Partitioning Studies—Ten milliliters of an aqueous phase containing alkaloid or alkaloid and caffeine was added carefully to 10 ml. of chloroform in a screw-capped vial. The vial was sealed, allowed to equilibrate for 1 min. in a water bath (30 ± 0.1°), and then rotated at 6 r.p.m. using the same apparatus as described for the solubility studies. Samples were taken from the aqueous phase and analyzed for alkaloid content by the Van Urk method (3).

Precipitation Studies—0.1 *N* sodium hydroxide was added to a solution of 10 mg. of ergotamine tartrate in 100 ml. of distilled water (30 ± 0.1°) at a rate which allowed a rise in pH of one unit/5 min. At each half pH unit a 10-ml. sample was withdrawn for analysis and filtered using a syringe fitted with a Millipore filter (0.45 μ). The sample volume was replaced with 10 ml. of a solution of equal concentration of ergotamine tartrate (0.01%) in distilled water. When the approximate precipitation range was found the experiment was repeated three times with samples taken at 0.2-unit increments in the precipitation range. Analysis was performed using the Van Urk method (3).

RESULTS AND DISCUSSIONS

The solubility of ergotamine tartrate varies with pH and, as can be seen in Figs. 1–3, the changes in the apparent solubility of ergotamine tartrate when caffeine is added are also a function of pH. At the pH of distilled water (5.5 in these experiments), maximum solubility is reached at a 30:1 molar ratio of caffeine to ergotamine tartrate (Fig. 1). The curve does not lend itself to analysis for determination of stoichiometry of the complex. The nature of solubilized species as indicated by Fig. 1 is such that a large number of caffeine molecules are required to solubilize each ergotamine tartrate molecule. This may indicate an environmental or cosolvent-like effect from the caffeine rather than complexation. In Fig. 2 the same observations apply. The molar ratio of caffeine to ergotamine tartrate at maximum solubilization is 20:1. At pH = 6.65 (Fig. 3) the molar ratio is approximately 800:1 at maximum solubilization. The maxima in the curve is probably due to saturation of the solution with respect to the solubilized species. The nature of the interaction between molecules is apparently dependent on the pH of the media and the species of ergotamine in solution at that pH. The

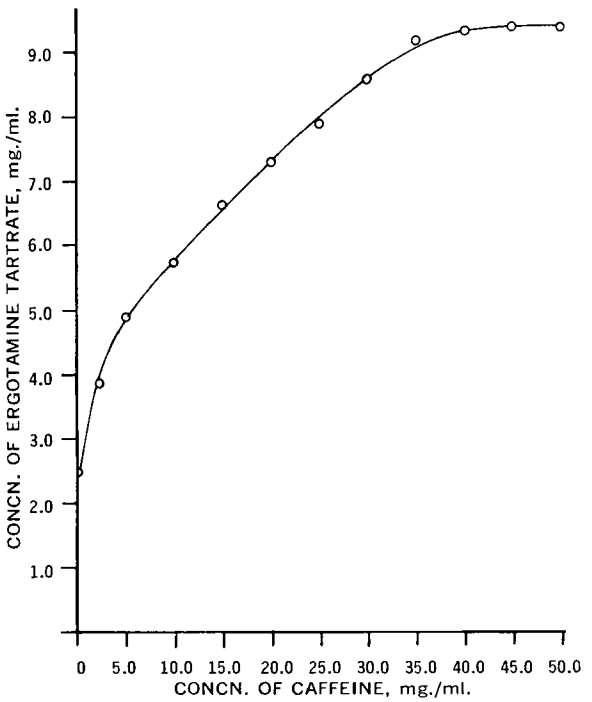

Figure 1—*Solubilizing action of caffeine on ergotamine tartrate in distilled water at 30°*

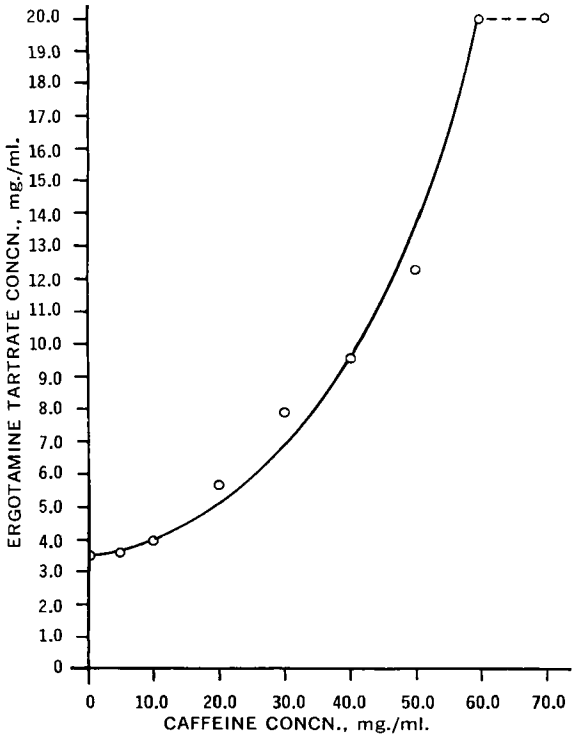

Figure 2—*Solubilizing action of caffeine on ergotamine tartrate in 0.1* N *hydrochloric acid at 30°*

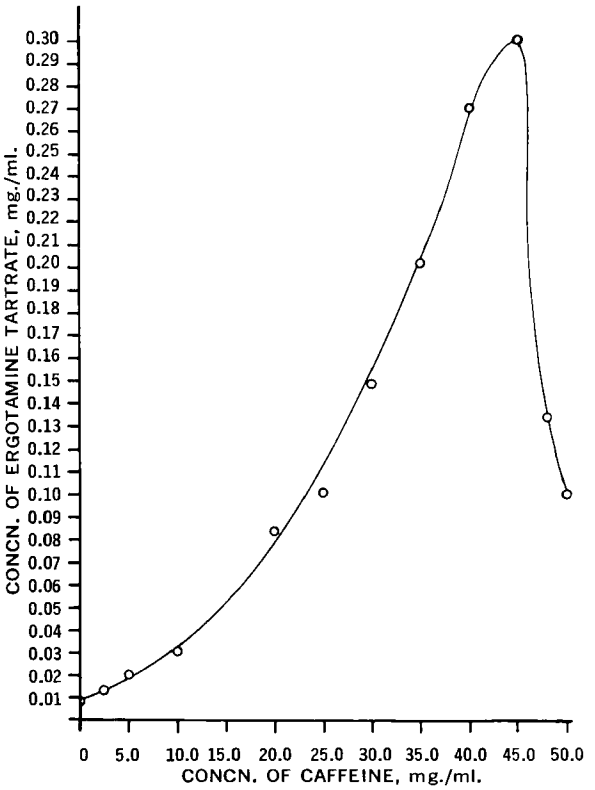

Figure 3—*Solubilizing action of caffeine on ergotamine tartrate in 0.1* M *phosphate buffer (pH = 6.65) at 30°.*

number of molecules of caffeine necessary to solubilize one molecule of ergotamine rises quite rapidly as the amount of ergotamine base present in solution is increased. In the studies at gastric pH the concentration of ergotamine base is zero. At pH = 5.5 the base would represent about 14% of the ergotamine in solution. At pH = 6.65 approximately 69% of the ergotamine would be in the basic form. With the solubility data and an apparent pKa value of 6.3 for ergotamine tartrate[1] in mind, the following approach was used to determine by *in vitro* experimentation the physicochemical fate of ergotamine when released from an oral dosage form containing the ergot alkaloid and caffeine combination:

1. The effect of complex formation on dissolution rate in simulated gastric fluid (0.1 *N* solution of HCl) was studied.

The dissolution rate, as seen in Fig. 4, was increased by a factor of three. The dissolution rate is considered by many to be the rate-limiting step in absorption (4–6). In this case, however, the very short time necessary to dissolve the ergotamine renders the caffeine effect noncontributing to the absorption picture unless the dissolution rate is inhibited by a particular dosage form. The pKa value of ergotamine indicates that at gastric pH most of the alkaloid would exist as the hydrochloride salt and the absorption of this ionic species is questionable (7).

2. The effect of increasing the pH of a solution of ergotamine tartrate was studied. One normal sodium hydroxide was added to a 0.01% solution of ergotamine tartrate until, at a pH of 6.2, the alkaloid precipitated. The precipitation could be prevented up to pH = 7.6 by the inclusion of 1% caffeine. At pH 7.6 the predominance of ergotamine base apparently causes precipitation.

3. Rates of partitioning and the effects of caffeine on these rates were studied with ergotamine tartrate at gastric and enteral pH.

The results at gastric pH and enteral pH are shown in Figs. 5 and 6. The data were found to follow first-order kinetics and are plotted in this manner. The slopes as given in Table I and as shown in Figs. 5 and 6 were calculated by the method of least squares from the experimental data. The 100:1 ratio of caffeine to ergotamine tartrate was chosen to correspond to the ratio in a commercial product.[2] If the curves can be considered to parallel absorption rates, solubilization would increase enteral absorption but hinder absorption from the stomach. The increased rate at enteral pH was

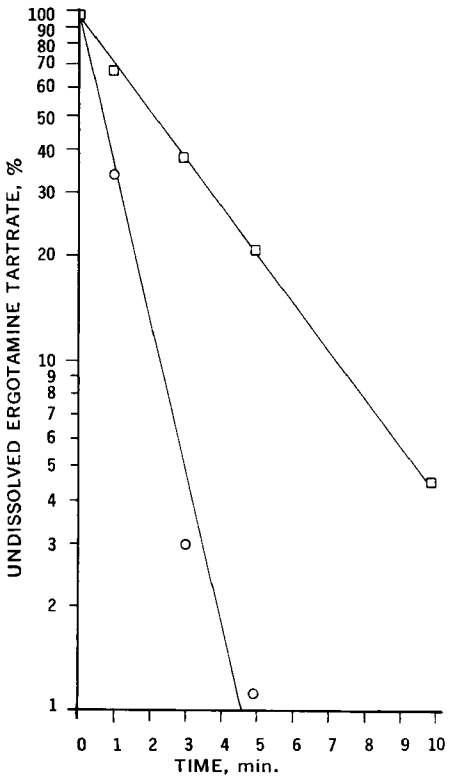

Figure 4—*Effect of caffeine on the dissolution rate of ergotamine tartrate. Key: □, dissolution rate of 50 mg. of ergotamine tartrate in 500 ml. of 0.1* N *hydrochloric acid; ○, dissolution rate of a mixture of 50 mg. of ergotamine tartrate and 5.0 g. caffeine in 500 ml. of 0.1* N *hydrochloric acid.*

[1] Determined by analysis of a solubility-pH profile for ergotamine tartrate. The same value was obtained potentiometrically utilizing a solution of ergotamine in 2% caffeine.

[2] Cafergot, Sandoz Pharm., Hanover, N. J.

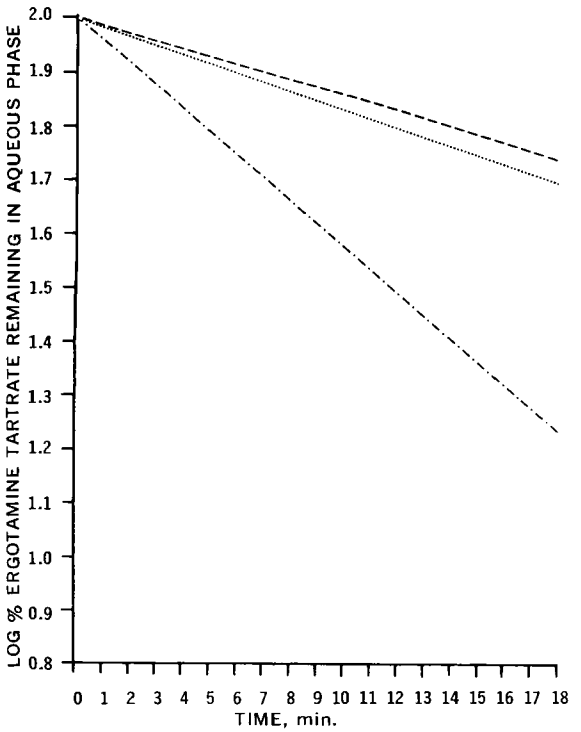

Figure 5—*Effect of caffeine on the partitioning rate of ergotamine tartrate from an aqueous phase (0.1* N *hydrochloric acid) to an organic phase (chloroform). Key: - - -, ergotamine tartrate (0.01 mg./ml.) plus caffeine (1.0 mg./ml.); · · ·, ergotamine tartrate (0.01 mg./ml.); — - —, caffeine (1.0 mg./ml.).*

probably not due to the transfer of a complex between phases, but rather due to the partial prevention of a precipitation step which occurred when ergotamine alone was present in the aqueous phase

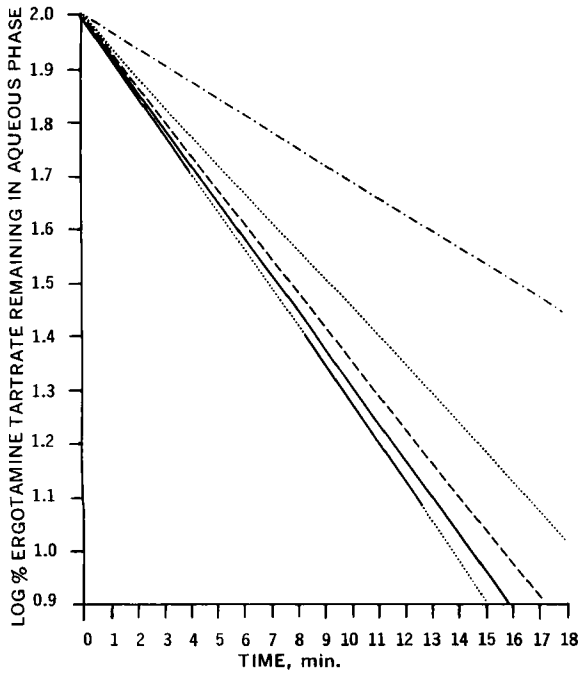

Figure 6—*Effect of caffeine on the partitioning rate of ergotamine tartrate from an aqueous (0.1* M *phosphate buffer pH = 6.65) to an organic phase (chloroform). Key: - — -, ergotamine tartrate (0.01 mg./ml.); · · ·, ergotamine tartrate (0.01 mg./ml.) plus caffeine (1.0 mg./ml.); - - -, caffeine (1.0 mg./ml.); ——, ergotamine tartrate (0.01 mg./ml.) [(samples filtered before assay using Millipore filter (0.45 μ)] plus caffeine (1.0 mg./ml.); · · · —, ergotamine tartrate (0.01 mg./ml.) [samples filtered before assay using Millipore filter (0.45 μ)].*

Table I—Summary of First-Order Partitioning Rates

Compound(s)	Rate
Partitioning from 0.1 N HCl to Chloroform	
Ergotamine tartrate (0.01 mg./ml.)	-1.6×10^{-2} min.$^{-1}$
Ergotamine tartrate (0.01 mg./ml.) and caffeine (1.0 mg./ml.)	-1.3×10^{-2} min.$^{-1}$
Caffeine (1.0 mg./ml.)[a]	-4.2×10^{-2} min.$^{-1}$
Partitioning from 0.1 M Phosphate Buffer (pH 6.65) to Chloroform	
Ergotamine tartrate (0.01 mg./ml.)	-3.2×10^{-2} min.$^{-1}$
Ergotamine tartrate (0.01 mg./ml.) and caffeine (1.0 mg./ml.)	-5.7×10^{-2} min.$^{-1}$
Ergotamine tartrate (0.01 mg./ml.)[b]	-6.9×10^{-2} min.$^{-1}$
Ergotamine tartrate (0.01 mg./ml.) and caffeine (1 mg./ml.)[b]	-6.5×10^{-2} min.$^{-1}$
Caffeine (1 mg./ml.)[a]	-7.3×10^{-2} min.$^{-1}$

[a] Determined alone and in the presence of ergotamine. [b] Samples filtered before assay using Millipore filter (0.45 μ).

(Fig. 6). This was shown by comparing the partitioning rate of ergotamine alone in experiments where the samples were filtered before assays to rates determined from unfiltered samples. The apparent rate obtained by filtering was faster and comparable to those obtained with unfiltered samples of ergotamine plus caffeine. The rates measured when ergotamine plus caffeine samples were filtered before assay was also faster than the rate calculated from the same experiment using unfiltered samples but almost identical to the rate of partitioning of the filtered samples of ergotamine alone. The partitioning rates thus point out the possibility of a precipitation step occurring before transfer of ergotamine to the organic phase which is partially prevented by the inclusion of caffeine. The rate of caffeine transfer in these experiments was determined with and without ergotamine present and found to be equal. The influence of pH on the rate of caffeine transfer is, however, quite noticeable in Figs. 5 and 6. The caffeine is apparently held more strongly in the acidic aqueous phase by interaction with hydrogen ion.

The combination of this interaction and the solubilizing interaction are both operative in affecting the partitioning rate of ergotamine and tend to hold back the ergotamine salt slightly. At neutral pH the caffeine is given up more freely by the aqueous phase due to its neutrality so that the only interaction affecting partitioning is the interaction between caffeine and ergotamine. The 500-fold concentration difference between the two compounds renders any solvent-caffeine interaction more likely to affect partitioning of the combination than a solvent-ergotamine interaction. The species present in solution apparently governs the rate of transfer of ergotamine. As the pH is increased and the amount of ergotamine base becomes greater the rate of partitioning is increased.

CONCLUSION

The total *in vitro* picture is as follows: the oral dosage form releases the combination of ergotamine tartrate and caffeine. The alkaloid is dissolved in the gastric fluid at approximately three times the rate with caffeine as it would without it. The influence of this increase of dissolution rate on absorption is questionable since the rate is extremely rapid with or without caffeine and also the pKa value of ergotamine indicates it would exist as an ionic species in the gastric fluid. As the alkaloid enters the small intestine it enters a pH region which would cause precipitation. Caffeine, as shown in the partitioning rate and pH studies, partially prevents this and keeps the drug in solution. The partitioning rate at enteral pH of the combinations of ergotamine tartrate and caffeine indicates bonding to be of such a nature as to freely release the complexed alkaloid to the organic phase. The effectiveness of the combination as viewed by the authors is due at least partially to complex formation or solubilization of ergotamine in a media where it would normally precipitate. This solubilization and the weak bonding of the complex in enteral media probably provide increased drug activity through ease of absorption.

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Evaluation of Antispasmodic and Related Activity in the Intact Dog

PHILIPPE S. BENOIT, NORMAN L. KATZ,* MAYER R. MINTZ, and HOWARD J. JENKINS

Abstract □ This is a report of the development of a method designed to assess concomitantly in the intact dog the antispasmodic activity of a compound, its effect on salivation, pupil size, heart rate, urinary activity, gastric acidity, gastric volume, blood pressure, and respiration. In the method the Me_{50} value of the antispasmodic is obtained graphically by plotting the response to each of three (or more) different postantispasmodic doses of methacholine chloride in terms of percent of the response to preantispasmodic dose(s) of methacholine. Such an Me_{50} value was obtained for each of three different parameters: motility of the digestive tract, motility of the urinary tract, and salivary secretory activity. The effects on other parameters which lend themselves to less exacting measurements are evaluated with considerably less precision. By supplying a means for comparing the effects of an antispasmodic compound on each of these three parameters under identical circumstances, the method makes possible the determination of the relative side-effect liability of that compound with respect to dryness of the mouth and urinary retention. Since the relative side-effect liability, expressed as a ratio, differs from one side effect to another and from compound to compound, the method provides an indication of the acceptability or usefulness of the compound.

Keyphrases □ Anticholinergic agents—bioassay method □ Bioassay method, anticholinergics—surgically intact dog □ Me_{50} value determination—anticholinergics □ Methacholine spasmodic activity—anticholinergic agent effect

The employment of anticholinergic agents in the therapy of the hypersecretory and hypermotile tract has dictated the adoption of various methods for the bioassay of such compounds. The majority of these represent *in vivo* methods such as those described by Ingelfinger (1) in 1943 and Code *et al.* (2) in 1952. For the most part these are involved with some sort of surgical manipulation of the animal, which may of itself alter the results obtained. In spite of the fact that the incidence of peptic ulcer is chiefly attributable to excessive secretion and/or motility, these methods have been concerned for the most part with the ability of the compounds undergoing potency testing to inhibit or abolish motor activity of the tract.

Since this is but one of the etiologic factors in the condition itself or the predisposition to it, and since the anticholinergic-type agent, being autonomic, exerts a variety of effects, the overall action of the agent in question becomes of considerable significance insofar as therapeutic usefulness is concerned. The toxicity of any drug is of great importance, but beyond this it is necessary to view the effectiveness of the drug against its side-effect liability. It goes without saying that the most effective drug is not necessarily the most useful. Accordingly a multiparameter assay of these anticholinergic agents which will assess not only antimotility and antisecretory activity but also side-effect propensity in the surgically intact dog is indicated if the whole action of these compounds in the body is to be projected accurately. Such an assay is necessarily adapted to the evaluation of neurotropic antispasmodic agents and in its development four agents in this category, available on the market, were selected for comparison with the neurotropic standard, atropine.

Turkanis (3) in 1963 described an assay method which may be used to compare the potencies of antispasmodic compounds in the surgically intact dog. The method was based on the establishment of a unit of antispasmodic activity: the Me_{50}, a computed quantity of methacholine, the response to which is reduced by the antispasmodic to 50% of the original value. This method, expanded and improved by Ryan (4) in 1964 and Benoit (5) in 1965, provided the basic antispasmodic procedure for this study. Since the work of Ryan had shown that the use of pentobarbital as an anesthetic might have an adverse effect on the results obtained, a combination of chloralose and sodium thiopental was employed in subsequent investigation.

EXPERIMENTAL

In the method presented, motility changes in the digestive tract are picked up by an inflated rubber sheath at the end of a polyethylene tube and transferred by the tube to a transducer and amplifier through which they are recorded. The rubber sheath is inserted