Cerebral Hemorrhage Associated With Phenylpropanolamine in Combination With Caffeine

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SUMMARY Phenylpropanolamine (PPA) is a drug that has been associated with serious side effects including stroke. It is often combined with caffeine in diet preparations and "look-alike" pills. In order to determine if PPA/caffeine can lead to stroke in normotensive and/or hypertensive rats, we administered the combination in six times the allowed human dose calculated on a per weight basis for the rats two times per day for five days. Subarachnoid and cerebral hemorrhage was noted in 18% of the hypertensive rats. A single PPA/caffeine administration (same dose) lead to acute hypertension in both the normotensive and hypertensive animals. These results suggest that PPA/caffeine can lead to cerebral hemorrhage in previously hypertensive animals when administered in greater than the allowed dosage. An acute elevation in blood pressure may be a contributing factor.

RECENT EVIDENCE SUGGESTS that the over-the-counter medication, phenylpropanolamine (PPA), can lead to stroke in certain individuals. Less severe complications include headaches, seizures, psychiatric disturbances and cardiac arrhythmias. Phenylpropanolamine is found in over-the-counter diet preparations and street "look-alike" pills, most of which contain PPA in combination with caffeine. "Look-alike" pills are made to look like prescription drugs, but do not contain the same ingredients. The purpose of this experiment was to determine if the PPA/caffeine combination could lead to stroke and/or an elevation in blood pressure in normotensive and hypertensive rats. The PPA/caffeine dose was calculated from the allowed dose for humans, taken x 6, and calculated to an equivalent dose/weight for the rats. There were two major experimental groups: 1. those repetitively injected with PPA/caffeine that were later examined histologically for brain abnormalities; 2. those in which blood pressure was recorded in awake animal before and after a single PPA/caffeine injection. Within each experimental group, half of the animals were drug treated and the other half were saline treated.

Methods Seventy-one rats were used for these experiments. Three different animal strains were studied: Sprague-Dawley normotensive rats (Nt), (Harlan Laboratories, Indianapolis, Indiana), spontaneously hypertensive rats (SHR) (Taconic Farms, Inc., Germantown, N.Y.) and stroke prone SHR (SP-SHR) (local colony obtained originally from Carl Hansen at NIH). The SP-SHR were on 1% salt in their drinking water in order to enhance elevation of blood pressure. All animals were male and between 6–8 months of age with the exception of the six experimental SHR (two female six months old and four male 12–14 months of age) used for repetitive doses.

Pure PPA (2-amino-1-phenyl-1 propanol HCl: Sigma Chemical Company) in combination with caffeine (anhydrous: Sigma Chemical Company) was used rather than the contents of commercial diet preparations (see diet pill analysis below). The PPA and caffeine were dissolved in saline (PPA 6 mg/kg; caffeine 24 mg/kg) and given intraperitoneally. The dose was six times that allowed for humans PPA 1 mg/kg; caffeine 4 mg/kg (see discussion). The PPA/caffeine was administered repetitively (observation of behavior and histologic examination) or once (blood pressure recorded).

Diet Pill Analysis Fourteen commercial over-the-counter tablets from five different packages and batch numbers were analyzed quantitatively for the presence of PPA/caffeine. Gas chromatography (Varian model 3700) in combination with a thermionic specific detector was used to analyze the samples against a known standard.

Administration of Repetitive Doses of PPA/Caffeine The repetitive doses of PPA/caffeine were given intraperitoneally two times per day at 9:00 a.m. and 1:00 p.m. for 5 days. During the repetitive doses, the animals were fasted (as though they were dieting) except one hour during the day when they were given Purina rat chow ad lib. Water was offered at all times. At the end of the repetitive dose treatment, the brain vasculature was flushed (see brain flush below) within three days (SHR) to one week (Nt plus SP-SHR).

Perfusion of the Brain Vasculature After Chronic PPA/Caffeine Administration for Histological Examination The animal was sedated with nembutal (40 mg/kg i.p.), the femoral artery was catheterized and blood
pressure was recorded. The animals were killed with one cc. of potassium chloride (i.a.). Immediately after death the ascending aorta was cannulated through the left ventricle and the descending aorta was ligated. The brain was quickly perfused through the cannula with 0.9% saline for three minutes in order to remove existing blood from the cerebral vessels. This was followed with 10% buffered formalin for three minutes for proper fixation of the tissue. The rat brains were allowed to fix in situ for approximately three hours before being carefully removed and placed in the 10% buffered formalin fixative. The brains were prepared for hematoxylin and eosin (H and E) histologic sections in the routine manner and read by one of us in blinded fashion.

Blood Pressure Recordings in the Awake Rat During PPA/Caffeine Administration

Before 10:00 a.m. the animal was anesthetized with methohexital sodium (brevital) (70 mg/kg). The femoral artery was catheterized and the arterial line was exteriorized at the back of the neck. The animal recovered from the sedation within approximately one-half hour. The same day, after 1:00 p.m., awake blood pressures were recorded before and two hours after a single intraperitoneal PPA/caffeine injection. Control animals were identically sedated, injected with saline and similarly recorded.

Results

The over-the-counter diet tablets were stated to contain 25 mg PPA and 100 mg of caffeine on the package. In 14 tablets, the PPA content was 14 ± 3% (mean ± SE) different than that stated on the package (range 0–32%). In the same pills, the caffeine content was 11 ± 2% different from that stated on the package (range 1–24%). Because the PPA/caffeine variability of commercial diet preparations was marked and because additives to diet pill preparations make them insoluble and difficult to put into solution, pure PPA/caffeine was used in these experiments.

When PPA/caffeine or saline was administered repetitively, animal behavior and physiology were grossly observed over the five days of treatment. In the normotensive and SHR group the experimental animals were generally more active within the first two hours after drug administration than the controls. Among the SP-SHR, the experimental animals acted dramatically different than the controls. Two experimental SP-SHR's were actively fighting the first day of the experiment and had to be separated. One of these animals died during the first night after only two PPA/caffeine injections (kidney tubular necrosis — see below). Another experimental SP-SHR died about 20 minutes after the third PPA/caffeine injection on the second day (cerebral hemorrhage — see below). On the third day, there were four remaining experimental animals. Of these, one was hyperactive, looked ill and had dried blood around his nose suggesting a possible hypertensive nosebleed. Another SP-SHR bled 1–2 cc of fresh blood per rectum on the fourth day.

![Abnormal Histology](http://stroke.ahajournals.org/)

FIGURE 1. The number of complications in normotensive (NT), spontaneously hypertensive (SHR) and stroke-prone SHR (SP-SHR) after repetitive saline injections (C = control) or 6 x the allowed dose of PPA/caffeine (T<sub>x</sub> = treated). The SHR and SP-SHR had subarachnoid hemorrhage (n = 3) and acute tubular necrosis (n = 1) noted during histologic examination of the brain and kidney.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Mean Arterial Pressure One Week After Last Repetitive Dose</th>
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<tbody>
<tr>
<td></td>
<td>MAP Control</td>
</tr>
<tr>
<td>NT</td>
<td>135±7 (n = 5)</td>
</tr>
<tr>
<td>SHR</td>
<td>163±11 (n = 5)</td>
</tr>
<tr>
<td>SP-SHR</td>
<td>193±9 (n = 6)</td>
</tr>
</tbody>
</table>

Values are mean ± SE. The arterial pressure was recorded in only 5 of the 11 control and experimental SHR studied.

*Refers to death of two experimental SP-SHR prior to the time the arterial pressure was recorded. Another SP-SHR was not recorded.
geal extravasation. When this brain was removed for histology, gross blood was noted on the basal surface of the brain. Another experimental SHR, a six month old female, demonstrated histologic subarachnoid hemorrhage over the base of the brain in several areas.

Of the 6 SP-SHR, two died spontaneously prior to the end of the experiment. The animal that died after two drug injections had extensive hemorrhage into the flank region bilaterally (about 3–5 cc of blood total) upon gross inspection. Histological examination of the kidneys revealed extensive acute tubular necrosis (fig. 1). The other SP-SHR died after the third drug injection. Histologic examination of the brain revealed multiple areas of recent hemorrhage including hemorrhage into the fornix and the roof of the third ventricle, as well as into the ventricle itself and the soft tissue of the velum interpositum (figs. 1 and 2).

The maximal blood pressure increase (fig. 3, table 2) attained after a single PPA/caffeine injection was 43 ± 7 mm Hg in the Nt animals (n = 6) and 22 ± 2 in the SHR rats (n = 7). In the two SP-SHR examined the pressure increase was 73 ± 1 mm Hg (202 ± 2 to 274 ± 1). Controls showed a negligible increase in blood pressure (fig. 3, table 2) [Nt: Δ4 ± 4 (n = 4), SHR: Δ3 ± 3 mm Hg (n = 6)]. Two SP-SHR injected with saline did not demonstrate an increase in pressure (Δ −9 ± 4, 172 ± 5 to 163 ± 4). The increase in blood pressure in all three groups was significantly elevated over their original blood pressure and over the saline treated groups (p < 0.001).

Discussion

The results of these experiments indicate that repetitive administration of PPA/caffeine at six times the allowed dose per weight can lead to cerebral hemorrhage (and tubular necrosis) in spontaneously hypertensive and stroke-prone SHR rats. A single PPA/caffeine administration leads to an acute increase in blood pressure in both the normotensive and hypertensive rats. There was not a direct correlation between the increase in the acute blood pressure elevation and cerebral hemorrhage.

There are several comments that should be made concerning the approach used in this experiment. First, the histologic examination was performed in blinded fashion so that bias could not contribute to the interpretation of the histologic data. Second, controls were used in every experiment so that any methodological problem contributing to cerebral hemorrhage would be abated. Third, a mg/kg dose was used rather than mg/animal to negate differences between animals of different weights. Fourth, the concentrations used in this experiment (6 mg/kg PPA; 24 mg/kg caffeine) were elevated above allowed human doses (1 mg/kg PPA; 4 mg/kg caffeine). The Advisory Review Panel on Over-The-Counter (OTC) Miscellaneous Internal Drug Products to the FDA recommends that PPA is safe in doses of 37.5 mg two times per day in immediate release tablet or 75 mg every day in a timed release capsule. Thus, according to this recommendation, a 50

![Figure 2](image.png)

**Figure 2.** Hemorrhage into the fornix, roof of the third ventricle and the ventricle in an SP-SHR after repetitive PPA/caffeine administration.

![Figure 3](image.png)

**Figure 3.** The maximal change (Δ) in mean arterial pressure in normotensive (Nt) and spontaneously hypertensive rat (SHR) during saline injections (C = control) and 6 × the allowed dose of PPA/caffeine (Tx = treated). There were four C-Nt, six Tx-Nt, six C-SHR and seven Tx-SHR studied. The increase in mean arterial pressure was significantly elevated (p < 0.001) in the treated groups compared to controls.
kg woman could theoretically safely take almost 1 mg/kg of PPA in tablet form or greater than one mg/kg safely in capsule form. Caffeine is often present in the tablet [Example-Appedrine:PPA (25 mg), caffeine (100 mg)] or capsule [Example-Dexatrim:PPA (50 mg), caffeine (100 g)] in four times the PPA dosage. Thus, the allowed human dose is conservatively 1 mg/kg PPA. Since caffeine is often combined with PPA in many diet preparations in four times that quantity an accepted caffeine dosage can be interpreted to be 4 mg/kg.

Phenylpropanolamine is structurally similar to ephedrine and amphetamines and can elevate blood pressure. Caffeine has also been reported to elevate blood pressure when taken acutely. In this study, the arterial pressure of all three animal groups was significantly acutely elevated after the PPA/caffeine injection. The rise in arterial pressure was greatest in the SP-SHR (although only two animals were studied) and least in the SHR. Thus, there was not a direct correlation between the acute rise in arterial pressure and cerebral hemorrhage. Other factors such as preexisting brain damage, abnormal oxygen delivery and autoregulatory function may also be factors in producing cerebral hemorrhage in previously hypertensive animals when PPA/caffeine is injected.

In human studies, although ingestion of 25 mg of PPA did not elevate blood pressure in normal individuals, ingestion of 85 mg of phenylpropanolamine led to a significant increase in both systolic and diastolic blood pressure in all normal volunteers studied. In some cases, a dangerous rise in blood pressure was reported after ingestion of 85 mg of phenylpropanolamine. Since an acute increase in systemic mean arterial pressure can lead to loss of cerebral blood flow autoregulation and "breakthrough" of the blood-brain barrier, subarachnoid or intracerebral hemorrhage could result. These complications have been noted in humans after ingesting "look-alike" pills1 and diet pills (Dr. Barry M. Diskant, personal communication).

Our finding of tubular necrosis in one SP-SHR after only two doses of PPA/caffeine supports others who reported renal failure as a complication of human ingestion of phenylpropanolamine-containing diet pills. In one of these reports22 acute tubular necrosis was described in a previously healthy 25 year old man after an overdose of diet pills containing phenylpropanolamine caffeine.

Although cerebral hemorrhage was not observed in the experimental normotensive rats examined in this study, abnormalities secondary to PPA/caffeine in normotensive rats cannot be excluded with these data. This is because only a small number of experimental animals were included in this study and the incidence of cerebral hemorrhage would have had to be substantial to detect this complication using such a small number of animals. In addition, histologic changes occur only when physiologic function is markedly abnormal. More sensitive techniques of monitoring physiologic changes in vivo could reveal findings not appreciated using the present techniques.

In summary, repetitive elevated administration per weight of PPA/caffeine lead to cerebral hemorrhage in hypertensive rats. One can conjecture that hypertensive humans are also "at risk" when taking PPA/caffeine. First, although humans do not commonly take more than allowed doses of PPA/caffeine "individual variability" suggests that reactions to lesser concentrations and numbers of doses could occur. Second, since a large percentage of our hypertensive population are not aware that they have hypertension and hypertension is more common among overweight individuals susceptible to taking diet pills, hypertensive rats. One can conjecture that hypertensive humans are also "at risk" when taking diet pills or "look-alike" pills prior to the event.

Acknowledgments

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References


<table>
<thead>
<tr>
<th>Table 2</th>
<th>Maximal Mean Arterial Pressure Attained After a Single PPA/Caffeine (6 x Allowed Dose/Wt) Administration to the Awake Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td><strong>BP&lt;sub&gt;i&lt;/sub&gt;</strong></td>
</tr>
<tr>
<td>Nt</td>
<td>129 ± 3</td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>171 ± 12</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE. Nt = Normotensive; SHR = spontaneously hypertensive; **BP<sub>i</sub>** = initial mean arterial pressure; **BP<sub>max</sub>** = maximal mean arterial pressure; **Δ** = change in arterial pressure between **BP<sub>i</sub>** and **BP<sub>max</sub>**; **BP<sub>f</sub>** = final mean arterial pressure. *p < 0.001 compared to initial, †p < 0.001 compared to in control. All mean arterial pressures were obtained from 6-8 month males.
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