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(+)Amphetamine-Stimulus Generalization to an Herbal Ephedrine Product

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GLENNON, R. A. AND R. YOUNG. *(+)Amphetamine-stimulus generalization to an herbal ephedrine product.* PHARMACOL BIOCHEM BEHAV **65**(4) 655–658, 2000.—We have previously demonstrated that a (+)amphetamine stimulus generalizes both to (−)ephedrine and caffeine. Using rats trained to discriminate intraperitoneal (IP) administration of 1.0 mg/kg of (+)amphetamine (ED_{50} = 0.4 mg/kg) from saline vehicle in a standard two-lever drug discrimination procedure, the present investigation shows that the (+)amphetamine stimulus generalizes to (+)amphetamine (ED_{50} = 1.0 mg/kg) when administered via the intragastric (IG) route, and that (+)amphetamine appears about 2.5-fold less potent when administered via the IG route compared to the IP route. Likewise, (−)ephedrine (ED_{50} = 10.8 mg/kg) and caffeine (ED_{50} = 32.9 mg/kg) are also 2.5-fold less potent when administered via the IG route compared to their potency when administered via the IP route. The (+)amphetamine stimulus also generalizes to an IG-administered herbal preparation (i.e., Herbal XTC®; the herbal preparation possesses an approximate potency roughly comparable to what might have been expected on the basis of its reported ephedrine and/or caffeine content. These results demonstrate, for the first time, that an ephedrine-containing herbal preparation can produce a (+)amphetamine-like effect in animals. © 2000 Elsevier Science Inc.

Amphetamine Ephedrine Caffeine Stimulants Drug abuse Herbal dietary supplements

WE have long been involved in studying amphetamine-related drugs of abuse and in formulating structure–activity relationships for amphetamine-like actions. Recently, we have turned our attention to analogs of amphetamine that bear a substituent at the β-position (14,15). Ephedrine, a β-hydroxy N-methyl analog of amphetamine, can be used as a precursor for the clandestine synthesis of methamphetamine via chemical reduction, and as a precursor for the synthesis of methcathinone (also known as ephedrone), another central stimulant drug of abuse, via oxidation (12). Ephedrine also possesses stimulant properties of its own. For example, (−)ephedrine, a major constituent of ma huang or *Ephedra sinica* and other *Ephedra* species (3,9), produces locomotor stimulation in rodents (2,13), and both racemic ephedrine (10) and (−)ephedrine (15) produce amphetamine-like stimulus effects in rats trained to discriminate (+)amphetamine from vehicle. Rats have also been trained to discriminate crude ephedra extract (5), racemic ephedrine (6), and (−)ephedrine (14) from vehicle, and the (−)ephedrine stimulus generalizes to (+)amphetamine and other central stimulants such as cocaine, methcathinone, and caffeine (14). A (+)amphetamine stimulus also generalizes to caffeine [see (15) and discussion therein], and a caffeine stimulus has been shown to generalize to (+)amphetamine (11). Evidence even suggests that caffeine can enhance the effect of ephedrine when the two are administered in combination (6,8,15).

The past decade has witnessed the introduction and popularization of various "herbal dietary supplements" and other over-the-counter ephedrine-containing preparations. Over 100 of these products have been promoted for such uses as weight loss, body building, increased energy, increased mental concentration, increased sexual sensations, euphoria, and as alternatives to illicit street drugs (3). Since 1993, the FDA has received more than 800 reports of illness and injury that appear to be related to ephedrine-containing products; several deaths also have been reported (3). Major side effects from ephedrine-containing preparations seem to involve cardiovascular problems and CNS stimulation. Although it is difficult to estimate the popularity of these products, according to the lay press (1) the manufacturers of Herbal Ecstacy® (*sic*) (Glo-

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bal World Media Corp., Venice, CA) alone claim to have sold 150 million dose units prior to 1997. Many of these products fail to indicate their specific contents; however, most preparations typically contain ephedrine in the form of ma huang or *Ephedra*, plus a caffeine-containing herbal ingredient (3). One of the products that does label its contents is Herbal XTC® (GH Applied Technologies Inc., Fairfield, CT). Herbal XTC® contains 200 mg of ma huang per tablet and, according to the labeling, this represents approximately 18 mg of ephedrine. Labeling also indicates that a tablet of Herbal XTC® provides 55 mg of caffeine. A companion product, Herbal XTC Enhancer® contains 590 mg of ma huang extract (providing 59 mg of ephedrine) per tablet.

Even though intraperitoneal (IP) administration of certain of the constituents of herbal products (i.e., ephedrine and caffeine) produce (+)amphetamine-appropriate responding [e.g. (15)], suggesting that the herbal preparations might be capable of mimicking the stimulus effects of amphetamine, the herbal products are normally taken orally. The purpose of the present investigation was to determine if oral administration of either (−)ephedrine or caffeine would result in stimulus generalization when given to rats trained to discriminate IP-administered (+)amphetamine from vehicle. Oral (+)amphetamine was also examined for purpose of comparison. A second goal of this work was to evaluate the effect of oral administration of an herbal preparation; Herbal XTC® was selected for examination in the same animals.

METHOD

The animals used in this study were six of the original nine animals previously described in an earlier study investigating the effect of IP-administered ephedrine and caffeine (15). Male Sprague–Dawley rats, weighing 350–400 g at the beginning of the study, were housed individually and, prior to the start of the study, their body weights were reduced to approximately 80% of their free-feeding weight. During the entire course of the study, the animals' body weights were maintained at this reduced level by partial food deprivation; the animals were allowed drinking water ad lib in their home cages. Once the study began, animals were only fed immediately following the test or training session. The rats were trained (15-min training session) to discriminate intraperitoneal injections (15-min presession injection interval) of 1.0 mg/kg of (+)amphetamine from vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reward (i.e., sweetened milk) using standard two-lever operant equipment as previously described (15). Daily training sessions were conducted with (+)amphetamine or saline; on every fifth day, learning was assessed during an initial 2.5-min nonreinforced (extinction) session followed by a 12.5-min training session. For half of the animals, the left lever was designated the drug-appropriate lever, whereas the situation was reversed for the remaining animals. Data collected during the extinction session included responses per minute (i.e., response rate) and number of responses on the drug-appropriate lever (expressed as a percent of total responses). Animals were not used in the subsequent stimulus generalization studies until they made greater than 80% of their responses on the drug-appropriate lever after administration of (+)amphetamine, and less than 20% of their responses on the same drug-appropriate lever after administration of saline.

Tests of stimulus generalization were conducted in order to determine if the (+)amphetamine stimulus would generalize to oral (+)amphetamine, oral (−)ephedrine, oral caffeine, and oral Herbal XTC.® During this phase of the study, maintenance of the (+)amphetamine–saline discrimination was ensured by continuation of the training sessions on a daily basis (except on a generalization test day; see below). On one of the two days before a generalization test, half of the animals would receive (+)amphetamine, and half would receive saline; after a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original criteria (i.e., >80% of total responses on the drug-appropriate lever after administration of training drug, and <20% of total responses on the same lever after administration of saline), during the extinction session were excluded from the next generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the training sessions. Drugs were administered either by the intraperitoneal (IP) route or were intragastically (IG) administered using a feeding tube. The animals were allowed 2.5 min to respond under nonreinforcement conditions; the animals were then removed from the operant chambers and returned to their home cages. An odd number of training sessions (usually five) separated any two generalization test sessions. Doses of the test drugs were administered in a random order, using either a 15-min or 30-min presession injection interval, to groups of four to six rats. If a particular dose of a challenge drug resulted in disruption of lever pressing (i.e., no responding), only lower doses would be evaluated in subsequent weeks. Stimulus generalization was said to have occurred when the animals, after a given dose of drug, made 80% of their responses (group mean) on the (+)amphetamine-appropriate lever. It was considered that lever-pressing behavior was disrupted if the animals made fewer than five total responses during the entire 2.5-min extinction session. Where stimulus generalization occurred, ED_{50} values were calculated by the method of Finney (4). The ED_{50} doses are doses at which the animals would be expected to make 50% of their responses on the drug-appropriate lever.

Drugs

(−)Ephedrine HCl ([1R,2S]-(−)-2-[methylamino]-1-phenylpropan-1-ol HCl) and anhydrous caffeine were purchased from Sigma–Aldrich Corp (St. Louis, MO). S(+)Amphetamine sulfate was available in our laboratories from previous studies. Herbal XTC® (GH Applied Technologies Inc., Fairfield, CT) was purchased from a commercial retail vendor. The dark brown tablets of Herbal XTC® were finely ground using a mortar and pestle; each 56 mg of powder contained (according to information provided on the product label) 1 mg of ephedrine. The required amount of powder was suspended in saline prior to use. Solutions of drugs and suspensions of the herbal preparation were made fresh daily in 0.9% sterile saline, and agents were administered either via intraperitoneal injection or intragastric administration in a 1.0 ml/kg injection volume; the 30- to 45-mg doses of caffeine and the 672-mg and 764-mg doses of Herbal XTC® were administered in a 2-ml/kg volume. Where applicable, doses refer to the weight of the salt. Intraperitoneal injections were made 15 min prior to testing, whereas testing after intragastric administration involved a 30-min delay.

RESULTS

The results of the stimulus generalization studies are shown in Table 1. An ED_{50} dose was calculated for the six animals administered IP (+)amphetamine (ED_{50} = 0.4 mg/kg). (+)Amphetamine was examined at four IG doses ranging

from 0.5 to 2 mg/kg; following the highest IG dose of (+)amphetamine the animals made 91% of their responses on the (+)amphetamine-appropriate lever (ED_{50} = 1.0 mg/kg). (−)Ephedrine was examined at six IG doses ranging from 5 to 20 mg/kg (Table 1). Following administration of 17.5 mg/kg of (−)ephedrine the animals made 86% of their responses on the (+)amphetamine-appropriate lever. Mean response rates after administration of these drug doses were not appreciably different from the response rates obtained after the administration of the training dose of the training drug or saline (see Table 1). Administration of 20 mg/kg of (−)ephedrine, however, resulted in disruption of behavior; that is, four of the six animals failed to make a total of five responses during the entire 2.5-min extinction session; the two animals that did respond (response rates of 2.0 and 3.2 responses/min) made 100% of their responses on the drug-appropriate lever. Five IG doses of caffeine were examined, and the (+)amphetamine stimulus generalized to IG caffeine (ED_{50} = 32.9 mg/kg). Four different doses of Herbal XTC® were examined: 280, 560, 672, and 784 mg/kg; these concentrations represent (according to the product labeling) approximate ephedrine doses of 5, 10, 12, and 14 mg/kg. Following the highest Herbal XTC® dose, the animals made 83% of their responses on the (+)amphetamine-appropriate lever. Mean response rates after injection of caffeine or Herbal XTC® doses were not substantially different from the response rates obtained after injection of 1 mg/kg of (+)amphetamine or saline. The ED_{50} value calculated for Herbal XTC® is 535 mg/kg (or approximately 9.5 mg/kg based on labeled ephedrine content).

TABLE 1

RESULTS OF STIMULUS GENERALIZATION STUDIES WITH RATS TRAINED TO DISCRIMINATE (+)AMPHETAMINE FROM SALINE VEHICLE

Agent	Route*	Dose (mg/kg)	N†	% Drug-Appropriate Responding‡	Response Rate (Reponses/min)‡
(+)Amphetamine	IP	0.25	6/6	14 (4)	13.5 (1.4)
		0.5	6/6	63 (15)	14.2 (2.9)
		1.0	6/6	95 (2)	14.9 (1.1)
				ED_{50} = 0.4 (0.2–0.8) mg/kg	
Saline (1 ml/kg)	IP		6/6	5 (2)	13.8 (1.9)
Saline (1 ml/kg)	IG		6/6	8 (3)	12.7 (2.1)
(+)Amphetamine	IG	0.5	6/6	15 (4)	13.1 (2.3)
		1.0	6/6	48 (16)	13.9 (3.0)
		1.5	6/6	69 (12)	12.7 (1.8)
		2.0	5/6	91 (3)	9.6 (2.1)
				ED_{50} = 1.0 (0.6–1.5) mg/kg	
(−)Ephedrine	IG	5.0	6/6	10 (4)	12.7 (1.9)
		7.5	6/6	22 (7)	14.1 (2.7)
		10.0	6/6	44 (15)	11.9 (2.6)
		15.0	5/6	67 (10)	9.6 (1.6)
		17.5	4/6	86 (4)	10.9 (4.7)
		20.0§	2/6	—	
				ED_{50} = 10.8 (7.9–14.7) mg/kg	
Caffeine	IG	10.0	6/6	6 (2)	12.4 (1.0)
		20.0	5/5	12 (4)	11.1 (1.4)
		30.0	6/6	32 (9)	13.3 (2.1)
		40.0	5/5	61 (15)	10.3 (1.9)
		45.0	4/6	83 (5)	8.1 (2/6)
				ED_{50} = 32.9 (22.7–45.5) mg/kg	
Herbal XTC®	IG	280	5/5	14 (5)	14.1 (2.1)
		560	4/4	41 (12)	13.6 (1.5)
		672	4/4	65 (15)	9.2 (2.1)
		764	4/4	83 (4)	9.7 (3.1)
				ED_{50} = 535 (357–801) mg/kg	

*Route of administration; the intraperitoneal (IP) route employed a 15-min presession injection interval, whereas the intragastric (IG) route employed a 30-min presession injection interval.

†*N* = Number of animals responding/number of animals administered drug.

‡Data obtained during a 2.5-min extinction session. ED_{50} values are followed in parenthesis by 95% confidence limits.

§Both of the responding animals made 100% of their responses on the drug-appropriate lever; responses per min = 2.0 and 3.2.

DISCUSSION

Administration of IG doses of (+)amphetamine to animals trained to discriminate IP (+)amphetamine from vehicle resulted in stimulus generalization. Within the time constraints and training conditions used in the investigation, IG-administered (+)amphetamine was approximately 2.5 times less potent than IP-administered (+)amphetamine. Likewise, IG (−)ephedrine (ED_{50} = 10.8 mg/kg; Table 1) and IG caffeine (ED_{50} = 32.9 mg/kg; Table 1) were about 2.5 times less potent than IP-administered (−)ephedrine and caffeine (IP ED_{50} = 4.5 and 12.9 mg/kg, respectively) (15). The results confirm our earlier finding (15) that both (−)ephedrine and caffeine are capable of producing (+)amphetamine-appropriate responding. In addition, the present results demonstrate that both agents are capable of producing similar effects when administered via the IG route, but that both agents, like (+)amphetamine itself, are about 2.5-fold less potent when administered IG than when administered intraperitoneally.

The next question to be addressed was whether or not an herbal preparation that contains ephedrine can produce (+)amphetamine-appropriate responding when administered via the IG route. Herbal XTC® was selected for examination for two reasons: (a) the agent was not difficult to obtain from commercial sources, and (b) the product's packaging specified the major ingredients and their amounts. The data in Table 1 show that administration of 764 mg/kg of Herbal XTC® resulted in (+)amphetamine stimulus generalization; the ED_{50} dose (535 mg/kg of powdered Herbal XTC®) represents approximately 9.5 mg/kg of ephedrine. Because the ephedrine in the herbal preparation is derived from ma huang, and because only the (−)isomer of ephedrine is known to be naturally occurring in the plant (9), it is assumed that the ephedrine in Herbal XTC® is (−)ephedrine. If this is the case, the ED_{50} dose of Herbal XTC® represents a dose of ephedrine (i.e., 9.5 mg/kg) that is consistent with the ED_{50} dose of IG-administered (−)ephedrine itself (10.8 mg/kg). The herbal product also contains caffeine. The amount of caffeine present in the ED_{50} dose of Herbal XTC® is calculated to be approximately 30 mg/kg; interestingly, this is very similar to the ED_{50} dose of IG-administerd caffeine (i.e., 32.9 mg/kg). The results suggest that a 535 mg/kg dose of powdered Herbal XTC® provides sufficient ephedrine and/or caffeine to substitute for (+)amphetamine in (+)amphetamine-trained animals.

We (15) and others (6,8) have previously shown that ephedrine and caffeine can mutually potentiate one another's amphetamine-like stimulus effects. It would have been informative had we been able to address this issue in the present study. However, there can be considerable variation between the labeled alkaloid content and the actual alkaloid content of ephedrine-containing herbal preparations (7); without having assayed the specific amounts of ephedrine and caffeine in the preparation employed herein, it is not possible to comment on whether or not any synergy occurred in the present study. Lack of knowledge about the rates of absorption of ephedrine and caffeine combinations from the powdered mixture following oral administration further complicates this issue. Consequently, the quantitative results obtained with the Herbal XTC® preparation are probably less accurate than the qualitative results.

In summary, doses of (−)ephedrine and caffeine administered via the IG route substitute for IP-administered (+)amphetamine and both are 2.5-fold less potent by the IG route than by the IP route. The (+)amphetamine stimulus also generalized to IG-administered powdered Herbal XTC® and the calculated ED_{50} dose apparently contains an amount of ephedrine and caffeine that is consistent with the calculated ED_{50} doses of (−)ephedrine and caffeine when administered alone. As such, this is the first demonstration that a commercially available ephedrine-containing herbal preparation can produce an effect similar to that produced by (+)amphetamine in animals. On the basis of product labeling, the specific preparation examined in this study (i.e., Herbal XTC®) was about 1/10th as potent as (+)amphetamine under the constraints of the present assay conditions.

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