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Comparison Between Phenylpropanolamine and Structurally Related Compounds on Gastric Transit in the Rat

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HULL, K. M., R. ZANZILLARI AND T. J. MAHER. *Comparison between phenylpropanolamine and structurally related compounds on gastric transit in the rat.* PHARMACOL BIOCHEM BEHAV **46**(2) 411–414, 1993.—Our laboratory previously reported several pharmacological differences between phenylpropanolamine [PPA; (±)-norephedrine] and its structurally related compounds in regard to their activity on cardiovascular and appetite-suppressant parameters. The present study investigates the pharmacological differences between PPA, [1*R*,2*R*]-(−)-norephedrine [(−)-NOR], [1*S*,2*S*]-(+)-norephedrine [(+)-NOR], [1*R*,2*S*]-(−)-ephedrine [(−)-EPH], [1*S*,2*R*]-(+)-ephedrine [(+)-EPH], [1*R*,2*S*]-(−)-norpseudoephedrine [(−)-NORP], [1*S*,2*R*]-(+)-norpseudoephedrine [(+)-NORP], [1*R*,2*R*]-(−)-pseudoephedrine [(−)-PSE], and [1*S*,2*S*]-(+)-pseudoephedrine [(+)-PSE], as determined by their ability to inhibit gastric transit in the rat. (−)-Norephedrine was approximately three times more potent in inhibiting gastric transit than (+)-NOR ($p < 0.01$). As anticipated, the racemic mixture, PPA, demonstrated an ED_{50} (25.1 mg/kg) of approximately the mean of the ED_{50}s from the component enantiomers (14.7 and 47.0 mg/kg, respectively). Similarly, administration of 20 mg/kg of either (−)-EPH, (+)-EPH, (−)-PSE, or (+)-PSE significantly decreased gastric transit by 26% ($p < 0.01$), 12% ($p < 0.01$), 10% ($p < 0.01$), and 11% ($p < 0.01$), respectively. Administration of (−)-NORP and (+)-NORP were without effect. These data confirm and extend previous findings demonstrating pharmacological differences between PPA and its structurally related compounds.

Phenylpropanolamine Ephedrine Norephedrine Pseudoephedrine Norpseudoephedrine Gastric transit

THE racemic mixture of (±)-norephedrine [phenylpropanolamine (PPA)] is present in numerous over-the-counter cough-, cold-, and appetite-suppressant medications. Phenylpropanolamine has been shown to derive its mechanism of action via both direct and indirect actions at adrenoceptors depending upon the site of administration and the dose administered (22,23). Additionally, compounds that are structurally related to PPA [e.g., ephedrine (EPH), pseudoephedrine (PSE), or norpseudoephedrine (NORP)] tend to exhibit similar pharmacological properties, although the relative potency may differ between compounds.

Minneman et al. (16) suggested that PPA may act as a partial agonist based upon results that demonstrated PPA to possess low affinity and low intrinsic activity at α_1-adrenoceptors. Our laboratory reported that the cardiovascular responses associated with administration of PPA and its component enantiomers tend to be largely mediated by direct activation of α_1- and α_2-adrenoceptors (12,17–19). Some controversy still exists as to whether or not PPA is capable of directly stimulating β-adrenoceptors; however, our laboratory recently reported experiments that measured the production of cyclic adenosine-3′,5′-monophosphate in heart minces as a direct result of β-adrenoceptor stimulation and failed to detect any activation by either PPA, its component enantiomers [1*R*,2*R*]-(−)-norephedrine [(−)-NOR] and [1*S*,2*S*]-(+)-norephedrine [(+)-NOR], or [1*R*,2*S*]-(−)-EPH, [1*S*,2*R*]-(+)-NORP, and [1*S*,2*S*]-(+)-PSE (11).

In addition to its direct interaction with adrenoceptors, PPA's actions may also be attributed, in part, to indirect mechanisms mediated at catecholamine-containing neurons. For example, PPA has been reported to inhibit the uptake of [^{3}H]norepinephrine in murine cardiac tissue (14) and inhibit liver monoamine oxidase (25). Additionally, PPA has been demonstrated to weakly release [^{3}H]dopamine from rat synaptosomes (15).

The mechanism(s) by which PPA suppresses appetite remains unspecified. However, in addition to central mechanisms, it is believed that PPA and its stereoisomers may medi-

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ate a portion of their anorectic activity via the inhibition of gastrointestinal smooth muscle, thus resulting in increased gastric transport time (21) and gastric retention (2,10,24).

When in the presence of food, nutrient receptors, located in the stomach, are activated, resulting in the inhibition of food intake. Deutsch (4) demonstrated that the withdrawal of a portion of a digested meal from the stomach will result in the animal immediately consuming additional quantities of food. Therefore, inhibition of gastric emptying would presumably extend the contact of nutrients with the stomach and thus prolong satiety.

The present study investigates the differences in activity between the enantiomers of PPA, NORP, EPH, and PSE to alter gastric transit in the rat.

METHOD

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were obtained at 125-150 g and individually housed in suspended wire mesh cages with food (Purina #5001) and tapwater available ad lib. Animals were acclimated to our climate-controlled animal facility for at least 1 week prior to experimentation. All experimental protocols were approved by our Institutional Animal Use and Care Committee prior to commencement of the studies.

Drugs

PPA HCl (Rhoer), (−)-NOR HCl (Rhoer), (+)-NOR HCl (Rhoer), [1*R*,2*S*]-(−)-norpseudoephedrine HCl [(−)-NORP; Rhoer], (+)-NORP HCl (Amend), (−)-EPH HCl (Rhoer), [1*S*,2*R*]-(+)-ephedrine HCl [(+)-EPH; Rhoer], [1*R*,2*R*]-(−)-pseudoephedrine HCl [(−)-PSE; Rhoer], and (+)-PSE HCl (Rhoer) were injected in a volume of 1 ml/kg IP.

A 1% amaranth (Sigma Chemical Co., St. Louis, MO) in 1% gum arabic (Sigma) "meal" was administered by oral intubation in a volume of 1 ml/animal 40 min prior to sacrifice.

Gastric Transit

Groups of five rats, weighing between 135-160 g, were fasted for 24 h. On the day of the experiment, animals received an IP injection of either PPA (10-30 mg/kg), (−)-NOR (5-25 mg/kg), (+)-NOR (25-100 mg/kg), (−) EPH (20 mg/kg), (+)-EPH (20 mg/kg), (−)-PSE (20 mg/kg), (+)-PSE (20 mg/kg), (−)-NORP (20 mg/kg), (+)-NORP (20 mg/kg), or saline (SAL) 60 min prior to sacrificing. These doses were chosen based upon previous studies indicating moderate anorectic activity (9). Twenty minutes later, 1 ml of a standard meal containing 1% amaranth in a 1% aqueous gum arabic solution was administered intragastrically by oral intubation (20). Forty minutes following the meal, animals were sacrificed by decapitation and the abdominal cavity exposed. Ligatures were placed at the esophageal and pyloric sphincters, to confine the stomach contents, and the small intestine (duodenum to the ileocecal valve) was carefully removed and the omentum sufficiently separated to enable unfolding of the intestine. Special care was taken to avoid stretching. The total length of the intestine and the distance the dye traversed were recorded to the nearest millimeter. Gastric transit is expressed as the mean percentage (± SEM) of the distance the dye traversed in relation to the total length of the small intestine (20). Data was analyzed via analysis of variance (ANOVA) and Dunnett's test.

RESULTS

PPA (10, 20, and 30 mg/kg) and its component enantiomers (−)-NOR (5, 15, and 25 mg/kg) and (+)-NOR (25, 50, and 100 mg/kg) produced a significant ($p < 0.05$) dose-dependent inhibition of gastrointestinal transit (total length of dye traversed vs. total length of small intestine) when com-

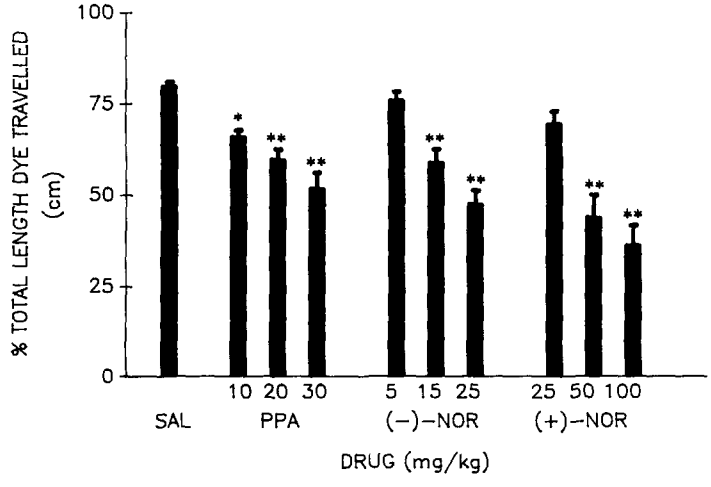

FIG. 1. Decreases in gastric transit induced by phenylpropanolamine (PPA), (−)-norephedrine (NOR), or (+)-NOR. Rats weighing between 135-160 g were administered either PPA (10-30 mg/kg), (−)-NOR (5-25 mg/kg), or (+)-NOR (25-100 mg/kg) and 20 min later administered 1 ml 1% amaranth in a 1% aqueous gum arabic solution. Animals were sacrificed 40 min later and the total length of the intestine and the distance the dye traversed were recorded to the nearest millimeter. Data is expressed as percent total length dye traveled. $^*p < 0.05$, $^{**}p < 0.01$.

pared to SAL control animals (79.5 ± 1.3%). At each of the doses tested, PPA decreased gastric transit by 18% ($p < 0.05$), 25% ($p < 0.01$), and 35% ($p < 0.01$), respectively. Increasing doses of (−)-NOR resulted in decreased gastric transit by 5%, 26% ($p < 0.01$), and 39% ($p < 0.01$); similarly (+)-NOR inhibited gastric transit by 13%, 45% ($p < 0.01$), and 55% ($p < 0.01$), respectively (Fig. 1). The ED_{50}s for PPA, (−)-NOR, and (+)-NOR were 25.1, 14.7, and 47.0 mg/kg, respectively. The slopes of the calculated lines were not significantly different.

The enantiomers (−)-NORP (20 mg/kg) and (+)-NORP (20 mg/kg), stereoisomers of PPA, failed to significantly affect gastric transit (total length of dye traversed vs. total length of small intestine) as compared with SAL control animals (78.4 ± 2.7%). Alternatively, administration of 20 mg/kg of either (−)-EPH, (+)-EPH, or its stereoisomers (−)-PSE or (+)-PSE significantly decreased gastric transit time by 26% ($p < 0.01$), 12% ($p < 0.01$), 10% ($p < 0.05$), and 11% ($p < 0.05$), respectively (Fig. 2).

DISCUSSION

The results presented here clearly demonstrate the ability of PPA and its component enantiomers (−)-NOR and (+)-NOR to significantly inhibit gastric transit. Moreover, these results demonstrate a difference in potency between (−)-NOR and (+)-NOR as evidenced by the significant ($p < 0.01$) difference between the ED_{50}s, 14.7 and 47.0 mg/kg, respectively. The results confirm previous data from our laboratory that demonstrated that (−)-NOR was approximately nine times more potent than (+)-NOR in suppressing appetite in hyperphagic rats (12). Additionally, Johnson and Maher (13) demonstrated the ability of (−)-NOR to be a more potent agonist than (+)-NOR for vasoconstriction in the isolated rat caudal artery. Moya-Huff et al. (17) previously demonstrated the pharmacodynamic differences between these enantiomers in the cardiovascular system of the rat. Although no studies to date exist that characterized differences between the pharmacokinetic profiles of these enantiomers, numerous studies exist that document significant pharmacokinetic differences between enantiomeric compounds (1,5,13).

In light of the differences in potency between (−)-NOR and (+)-NOR, it is not surprising to observe that the potency of PPA, a racemic mixture of the two enantiomers, is approximately the average of the separate enantiomer potencies. The results presented here clearly supported this hypothesis as evidenced by the experimentally determined ED_{50} of PPA (25.1 mg/kg) vs. the theoretically calculated ED_{50} (the average of the two individual enantiomer ED_{50}s), 30.9 mg/kg.

Similar to the enantiomers of PPA, the (−)-EPH enantiomer appears to be a more potent agonist than its corresponding (+)-EPH enantiomer as evidenced by the greater percentage of gastric inhibition (26 vs. 12%, respectively). Additionally, these results support previous findings that demonstrate the ability of (−)-EPH to dose dependently inhibit gastric transit, as well as gastric emptying (10). However, there appeared to be no significant difference between the relative potencies between the enantiomers of PSE (Fig. 2).

The enantiomers of NORP were unable to produce a significant alteration of gastric transit at the doses tested in our study. Although NORP is a stereoisomer of NOR, there does appear to be a significant difference in their pharmacology. Eisenberg et al. (7) previously reported the ability of (+)-NORP to significantly enhance central locomotor stimulatory activity in rats while PPA was without effect. Further, it was demonstrated that (+)-NORP was one tenth as potent as (+)-amphetamine in producing enhanced central locomotor stimulatory activity. This finding confirmed and extended previous observations by other laboratories that demonstrated a lack of CNS stimulatory activity of PPA (3,8).

In summary, these data confirm and extend previous findings that demonstrate the greater potency of the (−)-NOR

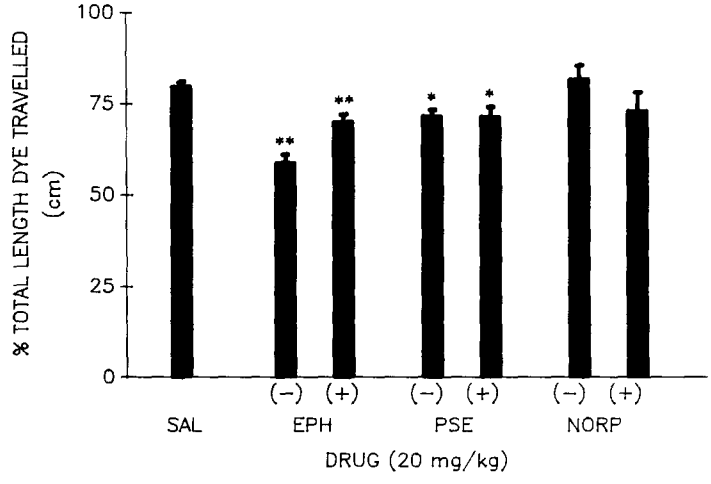

FIG. 2. Decreases in gastric transit induced by the (−)- and (+)-enantiomers of ephedrine (EPH), pseudoephedrine (PSE), and norpseudoephedrine (NORP). Rats weighing between 135–160 g were administered either 20 mg/kg of either (−)-EPH, (+)-EPH, (−)-PSE, (+)-PSE, (−)-NORP, or (+)-NORP and 20 min later administered 1 ml 1% amaranth in a 1% aqueous gum arabic solution. Animals were sacrificed 40 min later and the total length of the intestine and the distance the dye traversed were recorded to the nearest millimeter. Data is expressed as percent total length dye traveled. *$p < 0.05$, **$p < 0.01$.

enantiomer of PPA vs. the (+)-NOR in sympathomimetic-mediated actions and support the hypothesis that the effectiveness of PPA as an appetite suppressant may result from an action of both component enantiomers. Moreover, the enantiomers of EPH and PSE appear to be capable of inhibiting gastric transit in the rat, while the individual enantiomers of NORP failed to have an effect at the doses tested. Further studies investigating the pharmacological differences between PPA and its structural analogs are currently underway.

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