Effects of a False Neurotransmitter, p-Hydroxynorephedrine, on the Function of Adrenergic Neurons in Hypertensive Patients

ROBERT E. RANGNO, JOHN S. KAUFMANN, JOHN H. CAVANAUGH, DONALD ISLAND, J. THROCK WATSON, and JOHN OATES

From the Division of Clinical Pharmacology, Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37203

A B S T R A C T Previous studies have shown that amphetamine and p-hydroxyamphetamine impair adrenergic transmission, and it has been suggested that this effect is mediated by an active metabolite, p-hydroxynorephedrine (PHN). Studies in experimental animals have shown that PHN can deplete and substitute for norepinephrine (NE) in the transmitter pool, thus meeting the criteria of a false neurotransmitter.

The pharmacologic effects of PHN on adrenergic function and NE synthesis were studied in eight hypertensive patients and compared with placebo. Mean erect and supine blood pressure (BP) decreased 22/14 and 9/6 mm Hg, respectively, during PHN 600 mg daily. The post-Valsalva diastolic overshoot was abolished. The pressor sensitivity to tyramine decreased whereas the pressor response to NE was enhanced. A mild natriuresis occurred. The 24 h urinary excretion of catecholamines and catecholamine metabolites during the administration of PHN compared with placebo changed as follows: vanillylmandelic acid (VMA), 42% decrease; NE, 42% decrease; normetanephrine (NM), 400% increase; metanephrine, unchanged; dopamine, 40% decrease; while homovanillic acid was unchanged. The sum of VMA, NE, and NM decreased 23%. The post-treatment urinary excretion of PHN was biexponential with first and second phase half-lives of 13 and 55 h, respectively. The time of the second phase closely approximated the recovery of the changes in BP and excretion of VMA. No effects of PHN on the central nervous system were observed. These studies show that PHN acts peripherally to interfere with adrenergic function and NE synthesis in hypertensive patients with a resultant decrease in BP.

INTRODUCTION

Recent studies in this laboratory (1) have shown that amphetamine is metabolized to p-hydroxynorephedrine (PHN)[1] [d,l-α-(1-aminoethyl)-p-hydroxybenzylalcohol erythro configuration] in man, presumably by the pathway depicted in Fig. 1. The known ability of PHN to deplete norephinephrine (NE) (2) and to replace it in the neurosecetory stores (3) suggests that it may be an active metabolite which could interfere with adrenergic neuron function by acting as a false neurotransmitter. This possibility is given credence by evidence of impaired adrenergic neuron function after moderate to large doses of amphetamine (1). Also, p-hydroxyamphetamine (PHA), the intermediary metabolite in the conversion of amphetamine to PHN, has similarly been shown to suppress reflex sympathetic function in normotensive human (4) and to lower the blood pressure (BP) in hypertensive dogs (5).

The present study was initiated to determine whether PHN inhibited adrenergic function in man and to evaluate the possibility that introduction of this foreign amine might interfere with NE biosynthesis.

[1] *Abbreviations used in this paper:* BP, blood pressure; DA, dopamine; ECG, electrocardiogram; GLC-EC, gas-liquid chromatography with electron capture; HVA, homovanillic acid; M, metanephrine; NE, norepinephrine; NM, normetanephrine; PFP, pentafluoroproprionate; PHA, p-hydroxyamphetamine; PHN, p-hydroxynorephedrine; RBBB, right bundle branch block; VMA, vanillylmandelic acid.

METHODS

Design. Acute and subacute animal toxicity studies were performed initially and revealed no untoward effects of PHN within the dose range employed in this study (6). Clinical studies were conducted in the Vanderbilt Clinical Research Center after a complete investigation of the patients for hypertension and after their informed consent had been obtained. The study included eight patients, four male and four female, with a mean age of 41 yr (range: 36–59). The degree of hypertension ranged from moderate to severe. All patients had essential hypertension except for one patient (M. L.) with diabetes mellitus who had remained hypertensive after a nephrectomy for a unilateral renal artery stenosis. All antihypertensive medications had been discontinued at least 7 days in advance, and a 100 meq sodium diet was started on admission (one patient required a 10 meq sodium diet). The design included a single blind placebo period followed by PHN (*d, l*-erythro-*p*-hydroxynorephedrine) in oral, increasing, divided doses and a follow-up placebo period. Dose-ranging studies had been completed previously in three other patients. Doses were given every 6 h, and occasionally every 4 h. BP was measured in the supine and erect positions four time daily. BP was recorded three times at $\frac{1}{2}$-h intervals after any dose increment. Urine was collected daily in 10 ml of 10 N HCl for determination of sodium, creatinine, catecholamines, free catechol metabolites, and PHN. Urine samples were frozen before the analyses, except for NE determinations which were performed with fresh urine. Periodic assessment of routine hematologic, renal, and hepatic function was made initially, at the end of each dose increment, and after PHN was discontinued.

Pharmacologic tests. At the end of the initial placebo period, at the end of each effective PHN dose increment, and during the posttreatment placebo period, the following studies were performed: determination of the pressor sensitivity to the directly acting amine, norepinephrine, and to the indirectly acting amine, tyramine; determination of the depressor sensitivity to the alpha-blocking agent, phentolamine; and the Valsalva maneuver. The supine patient was prepared as follows: electrocardiogram(ECG)-monitoring leads were attached, indwelling intravenous and intra-arterial needles were placed, and a rest period of 15 min was allowed before the above studies. Test medications were given as single, rapid i.v. doses with sufficient time between doses to allow measurements to return to base-line values.

The dose of NE and tyramine required to raise the systolic pressure by 25 mm Hg was determined from the partial dose-response curve. At least three dose-response points were obtained for each test. The control dose was divided by that obtained during PHN administration, resulting in a ratio used to express the sensitivity to the pressor amine. The dose of phentolamine required to decrease the systolic pressure by 15 mm Hg was similarly determined. The ratio of control dose to treatment dose was used in this case to express the depressor sensitivity to phentolamine.

The Valsalva maneuver was performed by the supine patient maintaining a forced expiratory pressure of 40 mm Hg for 10 sec (7). The test was repeated at least two times on each occasion. The change in the pulse pressure during forced expiration was calculated as a percentage decrease of pretest pulse pressure. The change in the diastolic pressure in the immediate post-Valsalva period was calculated as the percentage increase of the pretest diastolic pressure.

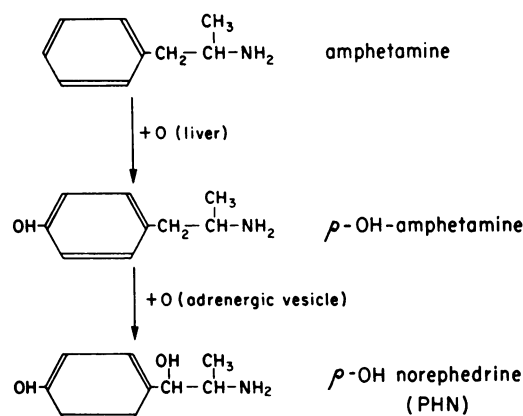*Urinary analyses.* (*a*) Vanillylmandelic acid (VMA)

FIGURE 1 Biotransformation of amphetamine.

was measured spectrophotometrically after its conversion to vanillin (8). The presence of large concentrations of PHN in the urine did not interfere with this assay.

(*b*) Free NE was measured fluorometrically (9). The presence of PHN in the urine caused a slight quench in the assay which did not exceed 20%. Correction for quench was made by the use of internal standards.

(*c*) Normetanephrine (NM) and metanephrine (*M*) were measured by a modification of the assay of Anton and Sayre (10). This assay was altered to include a further paper chromatographic separation of NM and M from PHN since large concentrations of PHN gave both quench and intrinsic fluorescence. The overall recovery through this procedure, determined in every sample by the recovery of tracer amounts of [^{3}H]NMN and [^{3}H]MN, was about 50%. The specificity of the separation procedure was verified by gas-liquid chromatography with electron capture (GLC-EC) plus mass spectrometry of a pentafluoropropionate (PFP) derivative of the NM sample.

(*d*) Dopamine (DA) was measured fluorometrically (11). The final eluate from this procedure was assayed for possible interfering PHN by PFP derivatization and analysis on GLC-EC. The sensitivity of this method was 50 pg of PHN and verified the absence of PHN in the final eluate.

(*e*) Homovanillic acid (HVA) was measured fluorometrically (12).

(*f*) PHN was measured spectrophotometrically after its conversion to *p*-hydroxybenzaldehyde (13). Sensitivity of the assay was 0.5 µg/ml. After acid hydrolysis the sample was adsorbed on an Amberlite CG-50 resin column (Rohmand Haas Co., Philadelphia, Pa.). The column was eluted with 4 N NH₄OH. A 4 ml sample of the eluate was oxidized with sodium metaperiodate and the UV absorption measured at 333 nm. Non-acid-hydrolyzed samples were assayed to determine the unconjugated (unchanged) portion of the total urinary PHN. Qualitative and quantitative verification of some samples was made with GLC-EC analysis of the PFP derivative of PHN.

Statistical analysis. All statistical analysis of data was performed using the Student's *t* test.

RESULTS

The effect of PHN on BP. Systolic BPs in the erect position decreased significantly in all patients during the administration of PHN 600 mg daily and the de-

TABLE I
BPs in the Erect Position (mm Hg)

Patient	Placebo	PHN 600	PHN 900	Placebo
		mg/day		
C. H.	149/100	129*/83*		148/102
M. L.	149/108	116*/87*		141/107
R. W.	138/103	114*/88*		137/98
D. K.	160/96	143*/97	149*/98	175/96
O. D.	150/107	137*/106	135*/97*	149/119
F. P.	175/141	135*/108*	130*/101*	176/132
B. D.	151/109	137*/99*	149/108	165/116
J. S.	163/120	146*/107*	136*/95*	164/114
Mean decrease from placebo		22.3/13.8	20.0/15.2	

All values are means of four measurements daily for at least 4 consecutive days.
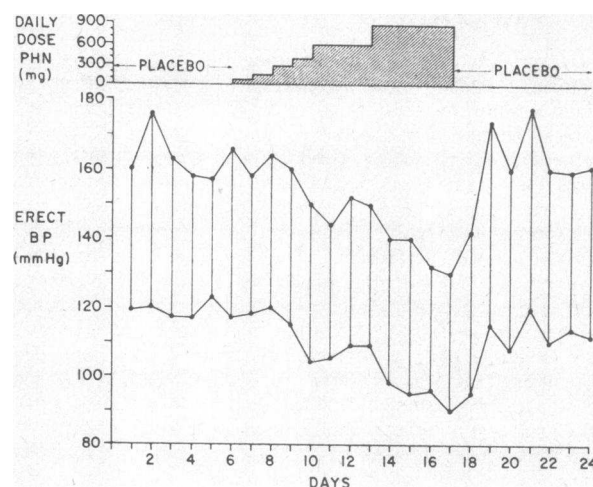* Differences between placebo and PHN significant $P < 0.05$ with majority $P \ll 0.01$.

FIGURE 2 BP change in one patient (J. S.) during PHN. The erect systolic and diastolic pressures decreased significantly ($P < 0.01$) during PHN 600 mg daily with a further decrease during PHN 900 mg daily.

crease in diastolic pressures was significant in seven of the eight patients. The mean decrease was 22.3/12.8 mm Hg compared with placebo (Table I). The pressure of three patients fell to normotensive levels on PHN 600 mg daily and this was their maximum dose. The dose was increased in the remaining five patients to 900 mg daily with little further change in supine and erect pressures (see Tables I and II).

All pressures returned to initial placebo levels during the post-PHN placebo period within 3–6 days after discontinuing PHN. The patients (F. P. and B. D.) received a short trial of PHN 1500 mg daily with no fur-

TABLE II
BPs in the Supine Position (mm Hg)

Pateint	Placebo	PHN 600	PHN 900	Placebo
		mg/day		
C. H.	142/89	123*/76*		130/77
M. L.	160/108	148*/103*		156/103
R. W.	138/94	133/87*		135/87
D. K.	164/93	162/98	167/96	181/97
O. D.	157/101	146*/107	144/99	148/105
F. P.	196/138	181*/127*	177*/115*	189/131
B. D.	166/105	164/103	168/110	179/109
J. S.	177/119	170/112	159*/102*	182/118
Mean decrease from placebo		8.5/5.6	9.0/6.4	

All values are means of four measurements daily for at least 4 consecutive days.
* Differences between placebo and PHN significant $P < 0.05$ with majority $P \ll 0.01$.

ther decrease in pressure. Symptoms related to postural hypotension were not observed during treatment with PHN. Systolic and diastolic BP in the supine position decreased significantly in four of the eight patients during PHN 600 mg daily compared with control (Table II). The mean decrease was 8.5/5.6 mm Hg compared with placebo. Of the patients who progressed to PHN 900 mg daily, only two of five showed a further decrease in supine pressure. Again, all pressures in the supine position returned to, or near to, control levels within 3–6 days after discontinuing PHN. An example of the course of one patient (J. S.) during the administration of PHN is shown in Fig. 2. There was no significant change in pulse rate during the study.

The effect of PHN on other indices of sympathetic function. Fig. 3 is representative of the effect of PHN on the Valsalva maneuver of one patient. During PHN, the decrease in pulse pressure was essentially unchanged, while the sympathetically mediated
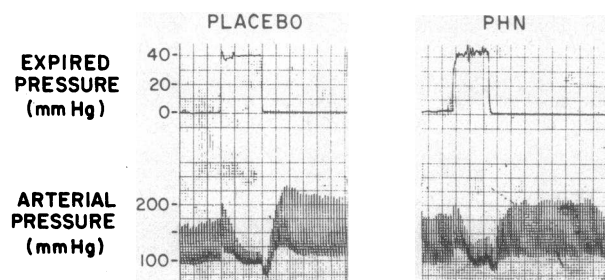

FIGURE 3 Valsalva's maneuver during placebo and PHN. A forced expiratory pressure of 40 mm Hg was sustained for 10 sec. The changes in the arterial pressure and their significance are described in the text.

diastolic overshoot was virtually abolished. A composite of the data from Valsalva maneuvers in all patients is shown in Fig. 4. Plots of the pre- and post-PHN data show a normal decrease in pulse pressure and reflex increase in diastolic pressure. During PHN 600 mg daily the decrease in pulse pressure was unchanged but reflex rise in diastolic pressure during the overshoot was substantially decreased in all patients to levels that fall below an established norm (7). These changes are similar to those seen after sympathectomy.

The pressor response to tyramine, an indirectly acting amine, and the pressor response to directly acting exogenous NE during placebo and PHN 600 mg daily are shown in Fig. 5. The pressor sensitivity to tyramine decreased markedly in all patients during PHN: that is, a larger dose of tyramine was required to raise the systolic pressure by 25 mm Hg. Tyramine testing was not carried out in all subjects because it appeared that tyramine transiently reversed the antihypertensive effects of PHN. The sensitivity to NE increased in five out of six patients during PHN; that is, a smaller dose of NE was required to raise the systolic pressure by 25 mm Hg.

The effect of PHN on the excretion of catecholamines and catecholamine metabolites. The effect of PHN on the 24 h urinary VMA is shown in Fig. 6. The progressive decrease in urinary excretion of VMA with increasing doses of PHN was highly significant; the maximum reduction was 57% at PHN 1,500 mg daily.

The effect of PHN on the 24 h urinary NM and M is shown in Fig. 7. NM increased about 400% above placebo levels during the administration of PHN 900 mg daily, and returned to normal in post-PHN placebo pe-
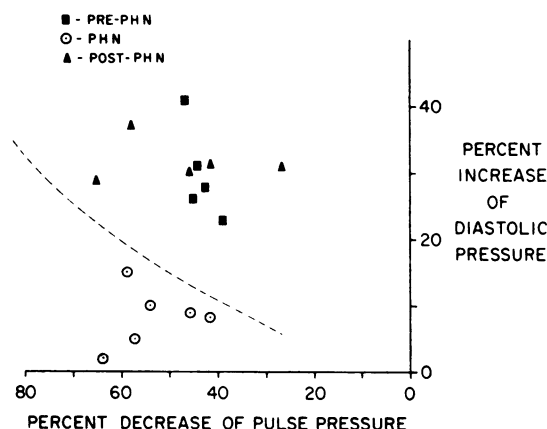

FIGURE 4 Results of Valsalva's maneuver in all patients studied. Horizontal and vertical axes are calculated according to Sharpey-Shafer (7). The dotted line represents the lower limit of normal according to Sharpey-Shafer. PHN dose: 600 mg daily during all data shown.
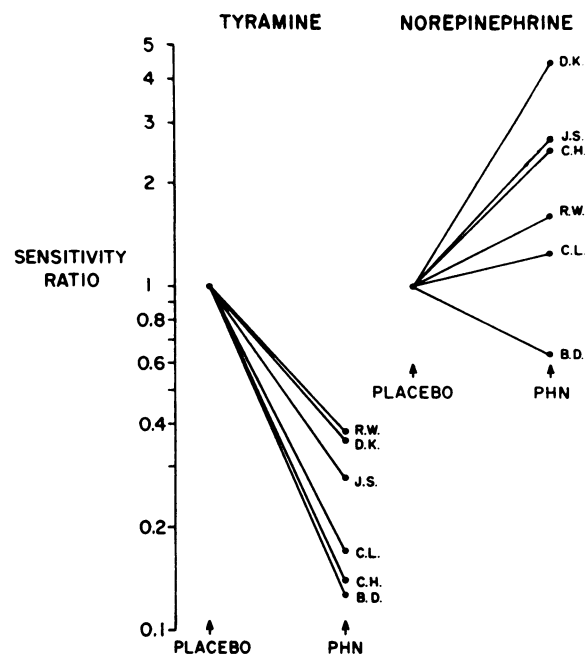

FIGURE 5 Pressor sensitivity ratios of tyramine and norepinephrine during placebo and PHN 600 mg daily. Sensitivity is expressed as the ratio of the dose of pressor amine required to raise the systolic pressure by 25 mm Hg during placebo compared with the dose required during administration of PHN.

riod. M, in contrast, remained unchanged in all patients studied.

The urinary excretion of NE is shown in Table III in conjunction with that of VMA and NM for these patients. The decrease in NE excretion was about the same percentage as the decrease in VMA. In order to evaluate the effect of PHN on the synthesis of NE, the sum of VMA, NM, and NE during PHN 600 mg was compared with these sums during the pre- and postplacebo periods. Total 24 h urinary excretion of the sum of VMA, NM, and NE decreased 23% during PHN administration.

In the four patients studied, urinary DA decreased significantly at all dose levels of PHN with a maximum decrease of 50% at PHN 1,500 mg daily, compared with pre-PHN placebo (Fig. 8). Again, values returned to near normal levels in the post-PHN placebo period. The urinary excretion of HVA, the metabolite of DA, was measured in the same four patients. The mean 24 h urine value during pre-PHN placebo was 2.77±0.26 mg/g creatinine and did not change significantly during PHN 600 or 900 mg daily or during post-PHN placebo.

The fate of PHN. Substantial oral absorption of PHN was verified by the urinary excretion of approximately 60% of the total administered dose as unchanged plus conjugated PHN. This percentage was similar at all dose levels. About 50% of the urinary PHN was un-
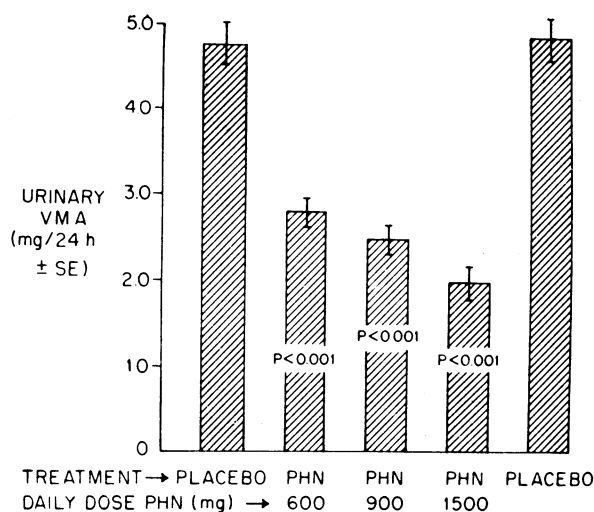
FIGURE 6 Effect of PHN on the 24 h urinary excretion of VMA. Values are the means ±SE of consecutive daily collections during the last 3 days of each period. Of the eight patients, the following numbers completed each treatment period: pre- and post-PHN placebo and PHN 600 mg: 8/8; PHN 900 mg: 5/8; PHN 1500 mg: 2/8. Statistical comparison of means is between pre-PHN placebo and each PHN dose period.

There was no evidence of psychic depression or stimulation at any level of PHN dosage. These observations were verified by a blinded psychiatric evaluation. A continuing review of systems failed to elicit any other untoward effects, except for the possibility of mild constipation in one female. Sexual function was difficult to assess in these hospitalized patients but direct questioning suggested no change in libido and two of the male patients on short leave during PHN administration described normal potency.

With the exception of one patient, there was no significant pressor effect during initial dosing with PHN 100 mg. The change in mean systolic pressure from time zero to ½, 1, and 1½ h post-PHN (or placebo) was + 6, + 7, and + 5 mm Hg during placebo, and − 2.4, + 6, and + 5 mm Hg during PHN. Single large doses of PHN in excess of 100 mg produced a mild transient increase in BP of 30/5 mm Hg in one patient when PHN was initiated at this dose level. After 3 days, doses in excess of 100 mg did not cause a pressor change in this patient. Single doses as large as 300 mg were tolerated by other patients without pressor effect; however, initial doses in these patients were never in excess of 100 mg.

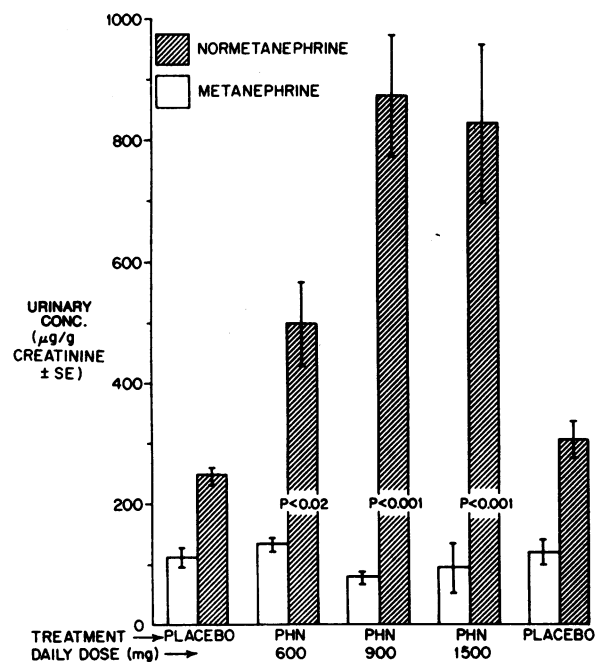An abnormality of ECG occurred in one patient (F. P.) during PHN. This patient had the most severe

changed and 50% was conjugated. The percentage of conjugated PHN was constant at varying doses of PHN. The time-course of urinary excretion of free plus conjugated PHN was studied in three patients during the post-PHN placebo period. The first phase of the PHN excretion had a mean $t\frac{1}{2}$ of 13 h while the second phase $t\frac{1}{2}$ had a mean of 55 h (Fig. 9).

The effect of PHN on sodium balance. A negative sodium balance was observed during PHN in each of four patients on a 100 mEq sodium diet, and the single patient on 10 meq sodium diet. Mean values for urinary sodium excretion during placebo, PHN 600 mg daily, and post-PHN placebo were 89.0, 114.4, and 82.6 meq/24 h, respectively. There was a 28% reduction in sodium excretion from the period of negative sodium balance during PHN to that of positive balance during post-PHN placebo ($P < 0.02$). The same mean values for urinary sodium for the single patient (F. P.) on a 10 meq sodium diet were 17.8, 19.1, and 4.6 meq/24 h. The positive balance from PHN to post-PHN placebo was again significant ($P < 0.01$).

Other evaluations. There were no significant changes in the following routine tests throughout this study: hemoglobin, hematocrit, white blood cell count, differential, platelets, fasting blood sugar, blood urea nitrogen, total bilirubin, serum glutamic oxaloacetic transaminase, uric acid, total proteins, alkaline phosphatase, serum sodium, potassium, chloride and bicarbonate, and urinalysis.

FIGURE 7 Effect of PHN on the 24 h urinary excretion of NM and M. Values are the means ±SE of at least three collections during the end of each treatment period. Of the three patients studied, the following numbers completed each treatment period: pre- and post-PHN placebo and PHN 600 mg: 3/3; PHN 900 mg; 2/3; PHN 1500 mg: 1/3.

hypertension, and required a 10 meq sodium diet through the placebo and PHN periods. She described chronic palpitations before and after the study. Before the study and during pre-PHN placebo, ECGs revealed left ventricular hypertrophy and first degree heart block as well as an intraventricular conduction defect. During PHN 600 and 900 mg daily, there was evolution of the conduction defect to right bundle branch block (RBBB). The patient noted palpitations but all vital signs were stable. Serial lactic dehydrogenase and serum glutamic oxaloacetic transaminase were unchanged. When PHN was discontinued, the RBBB reverted to an intraventricular conduction defect. At the time of her discharge, her hypertension was treated with a combination of hydrochlorothiazide, guanethidine, and methyldopa. Repeated ECGs during the post discharge period have shown periodic RBBB.

DISCUSSION

Although PHN is a minor metabolite of amphetamine, its formation after amphetamine administration occurs almost exclusively within adrenergic neurons; the primary metabolite of amphetamine, PHA, is actively transported into the neuron by the NE pump (14) and is β-hydroxylated intraneuronally (15). The administration of PHN to patients produces inhibition of sympathetic reflexes, as indicated by almost complete abolition of the pressor overshoot of the Valsalva reflex. These findings support earlier suggestions that alterations in sympathetic function produced by d-amphetamine (1) and p-hydroxyamphetamine (4) could be mediated by their active metabolite, PHN.

It is most likely that sympathetic reflexes are inhibited primarily at the level of the peripheral adrenergic neu-

FIGURE 8 Effect of PHN on the 24 h urinary excretion of DA. Values are the means ±SE of three collections during the each of each treatment period. Of the four patients studied, the following numbers completed each treatment period: pre- and post-PHN placebo and PHN 600 mg: 4/4; PHN 900 mg: 3/4; PHN 1500 mg: 2/4.

ron. A polar amine such as PHN should not readily cross the blood-brain barrier. Recent studies in our laboratory have shown catecholamines were depleted from adrenergic neurons in the rat heart by PHN, while brain catecholamine content was little altered. This is in

TABLE III

Urinary Catecholamines during PHN Compared with Pre- and Post-PHN Placebo

24-h urinary catecholamines* (μg/g creatinine)	Placebo	PHN (600 mg/day)	Placebo
VMA	3235±259	1932±181‡	3123±274
NM	277±24	773±81‡	267±37
NE	61±7	35±6‡	48±3
Total	3573	2740§	3438

VMA, vanillylmandelic acid; NM, normetanephrine; NE, norepinephrine.

* Values are the means ±SE of three separate 24-h urines in each period of treatment in the two patients in whom all these measurements were made (i.e., $n = 6$).

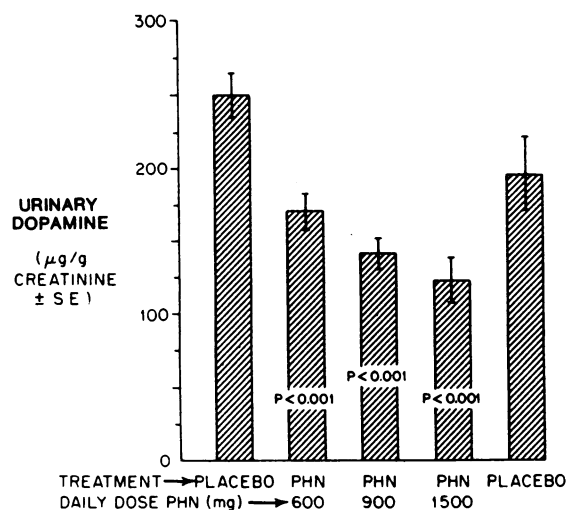‡ Differences between placebo and PHN significant ($P < 0.01$).
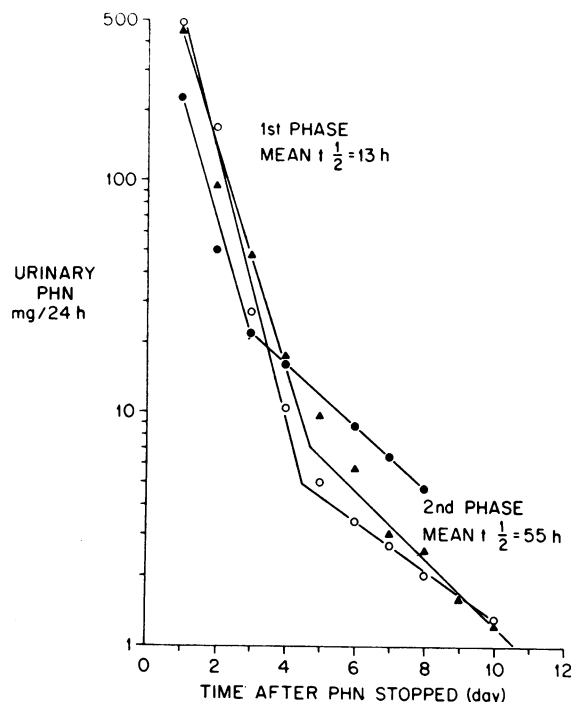
§ 23% decrease.

FIGURE 9 Delayed urinary excretion of PHN in three patients discontinuing PHN.

keeping with our clinical observations showing little discernible effect of PHN on the psychic function of the patients in this study. This is in marked contrast to the effects seen when false transmitters can be delivered into the brain in the form of an amino acid precursor such as methyldopa. Whereas a substituted phenylethylamine such as PHN may not easily enter the brain, it can be readily transported into the peripheral adrenergic neuron by the NE pump (14). Within the neuron, PHN can interfere with adrenergic function in at least two ways.

There is considerable evidence that it can replace NE within the neurosecetory vesicle from which it, in turn, can be released as a less potent "false neurotransmitter" (2, 3, 16). Another false neurotransmitter, metaraminol (*m*-hydroxynorephedrine) has also shown to have antihypertensive properties in man (17).

In our patients, NE depletion by PHN from the peripheral adrenergic neurons is suggested by the marked decrease in the pressor sensitivity to tyramine. The inhibition of tyramine's effect must be at the neuronal level because the slightly increased pressor response to exogenous NE indicates that the response of the receptor is not decreased. Part of the diminution in tyramine effect and the slight enhancement of NE effect could be due to competitive inhibition of tyramine uptake into the neuron by PHN. It is of interest that the diminished response to tyramine seen with PHN is in contrast to the enhanced response seen when the more potent alpha adrenergic agonist, alpha methylnorepinephrine, is the false transmitter (18).

In addition to replacement of NE within the secretory stores, the excretion of a diminished amount of catecholamines and total catecholamine metabolites suggests that the false neurotransmitter, PHN, also inhibited NE synthesis in these patients. The 23% decrease in the total measured metabolites of catecholamines is probably an underestimation of the decrease in NE synthesis because a fall in excretion of VMA in the presence of increased excretion of NM implies that the formation of the oxidative metabolite of the catecholamines, dihydroxymandellic acid, was also decreased. The decrease in excretion of both catecholamines and their metabolites is all the more remarkable in light of the concomitant fall in BP which alone is a stimulus for increased sympathetic activity and increased NE synthesis. These findings with PHN in man are in line with the observation that the related false transmitters, alpha-methylnorepinephrine and *m*-hydroxynorephedrine, cause a decrease in NE synthesis in the rat, as reported by Kopin, Weise, and Sedvall during the course of these studies (19). Because DA excretion was decreased and HVA excretion unchanged, it is most likely that the decrease in NE synthesis occurs before the beta hydroxylation step. A likely mechanism

would be release of NE from neurosecretory vesicles into the cytoplasm of the neuron where an increase in NE concentration would inhibit tyrosine hydroxylase (20, 21).

Because normetanephrine constitutes only a small fraction of total NE metabolites, total measured metabolites fell even though NMs excretion rose. This observed increase in catecholamine *o*-methylation would occur if NE uptake into the neuron (and exposure to intraneuronal oxidation) were blocked. Some inhibition of NE reuptake due to competitive inhibition of the NE pump by PHN is likely. There are similar changes in NM excretion when NE reuptake is blocked by imipramine (22). In light of the decrease in the pressor response to tyramine, partial inhibition of the NE pump is a more likely explanation of the increased normetanephrine excretion than is monoamine oxidase inhibition.

The observed interference with sympathetic control of arteriolar tone by PHN must be a major factor in the reduction of BP seen in these hypertensive patients in whom the fall in BP was greater in the upright than in the supine position. The increased excretion of sodium may also contribute to the antihypertensive effect, and is in contrast to the effect of guanethidine which causes sodium retention (23).

In these patients, the hypotensive dose range was 300–900 mg daily. The mild transient pressor effect noted in one patient when the dose was rapidly progressed to single doses of 100 mg or greater probably represents an indirect pressor effect; it did not occur on a second trial of the drug during which doses of PHN larger than 100 mg had no pressor effect after initial exposure to lower doses.

The urinary excretion of PHN is a biexponential function. The first phase (*t* ½ 13 h) represents excretion from a pool of relatively large mass, while the second phase (*t* ½ 55 h) represents excretion from a smaller pool which is more avidly retained at its site of extravascular storage. The time-course of disposition of PHN from this second pool closely parallels the disappearance of the hypotensive effect and the reversal of the changes in catecholamine synthesis and metabolism. This correlation with the effect on adrenergic neurons, the analogy with the neuronal storage of guanethidine (24), and the evidence for a neuronal pool of *m*-hydroxynorephedrine (25) all suggest that the slowly released pool represents that PHN stored in adrenergic neurons. The prolonged retention of this amine intraneuronally is facilitated by the alpha methyl group which confers relative protection from degradation by monoamine oxidase. The long half-life of the slowly released pool indicates that PHN will accumulate in it during continue administration of either amphetamine or PHN itself. Assuming that near maximal accumulation occurs after administra-

tion of PHN for about five half-lives, then its accumulation in this slowly released pool would continue for 11.5 days, reaching 87.5% of maximum levels after 7 days. When its precursor, amphetamine, is ingested on a continuing basis, maximal accumulation of PHN would not occur until 11.5 days after the several days required for maximal accumulation of amphetamine.

During amphetamine ingestion, the selective synthesis and storage of PHN in the adrenergic neuron and its accumulation to a greater extent than the parent drug suggests that a pharmacologic effect of this minor active metabolite would become maximal when amphetamine is ingested over many days as occurs with the chronic abuse or prolonged prescribed use of amphetamine. Indeed, in our earlier studies on short term ingestion of amphetamine, evidence for the effect of a false transmitter (e.g., decreased pressor response to tyramine) was not apparent until amphetamine had been taken for more than a day and even at 5 days there did not appear to be maximal inhibition of the pressor response to tyramine (1). These finds and the data on the half-life of PHN predict that the more extended ingestion of amphetamine should produce evidence of the effect of a false transmitter that is even more profound than observed in our earlier study on the short term ingestion of amphetamine. An evaluation of adrenergic function by measures such the pressor response to tyramine and the Valsalva maneuver in chronic amphetamine abusers would be of interest and could lead to a rapid diagnostic test for this condition.

When amphetamine is administered repeatedly, there is tachyphylaxis to its pressor effect (26). This has been confirmed in a recent study in animals which concluded that PHN might be involved in the tolerance to the peripheral but probably not to the central effects of amphetamine (27). This accounts for the fact that abusers of the drug can escalate the dose of amphetamine to amounts that would produce disastrous cardiovascular effects if taken as an initial dose. The diminished pressor response to another indirectly acting amine, tyramine, during continuing administration of either PHN or amphetamine suggests that depletion of NE by PHN is responsible for the tachyphylaxis of cardiovascular effects to amphetamine.

In summary, this investigation establishes that a peripherally acting false neurotransmitter such as PHN can interfere with adrenergic neuron function, an effect that is associated in hypertensive patients with a reduction in BP. The lack of central side effects is notable, and suggests that a compound capable of acting as a peripheral false transmitter might have therapeutic potential if the ratio of its pressor to catecholamine depleting effect were appropriately low.

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