Egan, R. W., Gale, P. R., Kuehl, F. A. (1979) J. Biol. Chem. 254: 3295–3302

Green, K., Samuelsson, B. (1964) J. Lipid Res. 5: 117–120

Kay, A. G. L., Griffiths, L. G., Volans, G. N., Grahame, R. (1980) Curr. Med. Res. Opin. 8: 548–551

Kean, F. A. (1968) Arch. Biochem. Biophys. 127: 528–533

Kean, F. A. (1970) Biochem. Pharmacol. 19: 2201–2210

Kuehl, F. A., Egan, R. W., Humes, J. L., Beveridge, G. C., Van Arman, G. C. (1977) Nature (London) 265: 170–173

Needleman, P., Raz, A., Ferrendelli, J. A., Minkes, M. (1977) Proc. Nat. Acad. Sci. U.S.A. 74: 1716–1720

Neuman, M. (1980) Drug Exp. Clin. Res. 6: 53–64

Panarelli, P., Berti, M., Gatti, M. T., Mosconi, P. (1980) Il Farmaco Ed. Sci. 35: 836–842

Piper, P. J., Vane, J. R. (1969) in: 'Prostaglandins, peptides and amines', Academic Press, London, 15–19

Raimondi, L. Banchelli, Soldaini, G., Buffoni, F., Ignest, G., Mussacesi, L., Amaducci, L., Friedmann, C. A. (1982) Pharmacol. Res. Commun. 14: 103–112

Regoli, D., Vane, J. R. (1964) Br. J. Pharmacol. 23: 351–359

Svensson, J., Hamberg, N., Samuelsson, B. (1975) Acta Physiol. Scand. 94: 222–228

Vane, J. R. (1957) Br. J. Pharmacol. 12: 344–349

Vane, J. R. (1971) Nature New Biol. 231: 232–235

J. Pharm. Pharmacol. 1983, 35: 264–265
Communicated September 27, 1982

0022–3572/83/040264–02 $02.50/0

In-vivo catecholamine formation from phenylephrine

JAN R. CROWLEY, CLYDE M. WILLIAMS*, MELVIN J. FREGLY, *Veterans Administration Hospital and Departments of Radiology and Physiology, University of Florida College of Medicine, Gainesville, Florida, 32610*

Exogenous phenylephrine (*m*-synephrine) is known to be metabolized by three major metabolic pathways: (1) conjugation as a sulphate (Bruce & Pitts 1968), (2) reduction to *m*-hydroxyphenylglycol (Rawlow et al 1980) and (3) oxidation to *m*-hydroxymandelic acid (Crowley et al 1981; Hengstmann & Goronzy 1982). Since phenylephrine can be converted to adrenaline by a non-specific hydroxylation enzyme system in liver (Axelrod 1963) we undertook to determine whether this alternate metabolic pathway occurred in-vivo and if so, to what extent.

Method

A 24 h control urine specimen was collected from 11 male Sprague-Dawley rats. Six of these were injected intraperitoneally with 300 μg of (±)-*N*-trideutero-methyl-*m*-synephrine HCl synthesized as described by Midgley et al (1980). The other 5 rats were injected with 0·9% NaCl (saline) solution. Twenty-four hour urine samples were converted to pH 1 with conc. HCl and heated at 95 °C for 1 h. After neutralization to pH 6 with 2 M NaOH, the mixture was passed through a strong cation exchange resin (AG 50W-X2, 100–120 mesh Bio-Rad). After washing the resin with water, the amines were eluted with 10 ml of 1 M NH_4OH in 65% ethanol. This eluate was then reduced to dryness by rotary evaporation and reconstituted in a small volume (1 ml) of 0·2 M ammonium acetate (pH 6) and placed on a weak cation exchange resin (Bio-Rex 70 200–400 mesh, 2 g). This column was washed with the ammonium acetate buffer and the 25–100 ml fraction containing phenylephrine and metanephrine was then passed through another column containing the cationic exchange resin (3 g) to remove the ammonium acetate buffer and the amines were eluted with 10 ml of 1 M NH_4OH in 65% ethanol. The amine fraction was then reduced to 100 μl by rotary evaporation, transferred to a 2 ml disposable vial and blown to dryness under N_2. The dried residue was treated with pentafluoropropionic acid anhydride (PFPA) for 15 min at 60 °C. The excess PFPA was evaporated under N_2 and the residue taken up in 25 μl of ethyl acetate. Of this, a 1 μl aliquot was injected into a g.c.-m.s. (Hewlett-Packard 5992A using a silanized glass column (1·8 m × 2 mm i.d.) packed with 5% OV-101 on Chromosorb-GHP 100/120 mesh (Supelco). The g.c. was operated isothermally at 220 °C using He as the carrier gas.

Identification of the PFP derivative of metanephrine was made by comparing the ratio of the base peak (*m/z* 190) to the molecular ion (*m/z* 635). The ratio of *m/z* 190/*m/z* 635 was 15 for authentic ($[^2H_0]$metanephrine standard (Regis Chemical Co.) and 15 for the biological unknown at the same retention time (4·4 min) in rat urine. After administration of $[^2H_3]$phenylephrine the same rat demonstrated peaks at *m/z* 193 and *m/z* 638 with the same ratio at the same retention time. The amount of $[^2H_3]$metanephrine is three times that of the endogenous $[^2H_0]$metanephrine found in the control urine specimens (Table 1). This provides unequivocal evidence for the presence in-vivo of the metabolic pathway phenylephrine → adrenaline → metanephrine after the intraperitoneal administration of phenylephrine.

Quantitative mass spectrometry may be carried out using deuterated analogues or chemical homologues. Since we could not use deuterated metanephrine as an internal standard, it was necessary to use a chemical homologue. For this purpose we chose $[^2H_0]$phenylephrine, which we had previously demonstrated does not occur naturally in rat urine in amounts > 1 ng mg^{-1} creatinine. The PFP derivative of phenylephrine has a retention time of 2·7 min and has a characteristic molecular ion *m/z* 605 and a base peak, *m/z* 190. After establishing that rats excreted no detectable phenyleph-

* Correspondence.

Table 1. Excretion of $[^2H_0]$metanephrine and $[^2H_3]$metanephrine after administration of 300 μg of phenylephrine.

Group (n)	Treatment	$[^2H_0]$metanephrine (ng day^{-1})	$[^2H_3]$metanephrine (ng day^{-1})	Total metanephrine $[^2H_0+^2H_3]$ (ng day^{-1})
1(5)	Control	311 (±68)	—	311 (±68)
	Saline Injection	426 (±139) NS	—	426 (±139) NS
2(6)	Control	302 (±44)	—	302 (±144)
	$[^2H_3]$phenylephrine Injection	778 (±348) +257% $P < 0.01$	1003 (±850)	1781 (±981) +589% $P < 0.005$

Each value represents the mean ± s.d.

rine, an internal standard of 1 μg of phenylephrine (Sterling Chemical Co.) was added to each 24 h urine sample. The amount of metanephrine was calculated by comparing the ratio of intensities of the ion *m/z* 190 at 2·7 min (phenylephrine) to the ion *m/z* 190 at 4·4 min (metanephrine) and relating this to the corresponding ratio on the calibration curve of 1:1 mixture of phenylephrine and metanephrine

Results

Table 1 shows the results of the intraperitoneal injection of 300 μg of $[^2H_3]$phenylephrine on the excretion of $[^2H_0]$metanephrine (endogenous) and $[^2H_3]$metanephrine (exogenous). Control rats excreted about 300 ng of $[^2H_0]$metanephrine daily. After the injection of 300 μg $[^2H_3]$phenylephrine, the rats excreted approximately 780 ng of $[^2H_0]$metanephrine and 1000 ng of $[^2H_3]$metanephrine. The combined total was approximately 6 times the normal amount of $[^2H_0]$metanephrine excreted by the controls. The increased excretion of $[^2H_0]$metanephrine in $[^2H_3]$phenylephrine-injected rats is significantly higher than the controls whereas the rise in $[^2H_0]$metanephrine in saline-injected rats is not significantly higher than controls. This increase in endogenous $[^2H_0]$metanephrine is probably due to release of stored adrenaline by the deuterated phenylephrine. The excretion of $[^2H_3]$metanephrine is attributed to the in-vivo conversion of $[^2H_3]$phenylephrine to $[^2H_3]$adrenaline followed by its metabolic inactivation by the enzyme catechol-*O*-methyl transferase.

The amount of $[^2H_3]$metanephrine excreted (1000 ng) is 0·33% (± 0·27%) of the amount (300 μg) of $[^2H_3]$phenylephrine administered. By comparison with the metabolic conversion of phenylephrine to *m*-hydroxyphenylglycol sulphate (50%), *m*-hydroxymandelic acid (6%) and phenylephrine sulphate (or glucuronide) (16%), the magnitude of this metabolic pathway for exogenous phenylephrine is minor. It is probable that the predominant site of formation of adrenaline from exogenous phenylephrine is in the liver because the ring-hydroxylating enzyme for this reaction is found there (Axelrod 1963). In the rat 70% of adrenaline is metabolized to metanephrine of which 20% is excreted in the urine as the glucuronide and 50% is deaminated and excreted as vanillylmandelic acid (Axelrod et al 1958). Therefore our estimate of 0·33% conversion of phenylephrine to adrenaline is a lower limit and in fact probably represents about 1·6% of the total adrenaline formed. Recently phenylephrine has been found to occur naturally in the adrenal gland (Midgley et al 1980; Durden et al 1980). The existence and magnitude of the pathway endogenous phenylephrine → adrenaline → metanephrine is not known.

Phenylephrine is widely used as a nasal decongestant. The amount administered to the rats was comparable on a body weight basis to the recommended amount used by an adult human for medicinal purposes. The metabolism of adrenaline in man is similar to that in rats. In man urinary metanephrine represents 40% of the total metabolism of adrenaline (LaBrosse et al 1961). Based on these results it is likely that man ingesting phenylephrine increases the formation of adrenaline by at least a factor of 15, of which at least half is contributed by release of endogenous adrenaline and the remainder by in-vivo ring hydroxylation of exogenous phenylephrine. Although the pressor effects of exogenous phenylephrine are undoubtedly due principally to their direct α-receptor agonist activity, there may well be, in addition, a smaller component contributed by increased adrenaline release or formation.

REFERENCES

Axelrod, J. (1963) Science 140: 499–500

Axelrod, J., Inscoe, J. K., Senoh, S., Witkop, B. (1958) Biochim. Biophys. Acta 27: 210–211

Bruce, R., Pitts, J. E. (1968) Biochem. Pharmacol. 17: 35–36

Crowley, J. R., Couch, M. W., Williams, C. M., Threatte, R. M., Fregly, M. J. (1981) Clin. Chim. Acta 109: 125–131

Durden, P. A., Juorio, A. V., Davis, B. A. (1980) Anal. Chem. 52: 1815–1820

Hengstmann, J. H., Goronzy, J. (1982) Eur. J. Clin. Pharmacol. 21: 335

LaBrosse, E. H., Axelrod, J., Kopin, I. J., Kety, S. S. (1961) J. Clin. Invest. 40: 253–260

Midgley, J. M., Couch, M. W., Crowley, J. R., Williams, C. M. (1980) J. Neurochem. 34: 1225–1230

Rawlow, A., Fleig, H., Kurahashi, K., Trendelenburg U. (1980) Nauyn-Schmiedeberg's Arch. Pharmacol. 314: 237–247