J. Pharm. Pharmacol. 1992, 44: 672–675
Received September 23, 1991

Urinary Excretion of Ephedrine After Nasal Application in Healthy Volunteers

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Abstract—The urinary excretion of ephedrine after intranasal administration of the drug was studied in 8 healthy volunteers. Ephedrine (6 drops of a commercial 0·75% nasal ephedrine solution in each nasal cavity) was administered 4 times at intervals of 2 h (total amount applied equivalent to approximately 14 mg ephedrine), and urine was collected each hour for 10 h; the volunteers exercised on a bicycle ergometer at 50% of their $\dot{V}O_{2max}$ for 2 h after the last ephedrine application. Ephedrine was detected in all urine samples. The urinary ephedrine concentration ranged from 0·9 to 16·5 $\mu g\ mL^{-1}$; the number of urine samples with an ephedrine concentration exceeding 5 $\mu g\ mL^{-1}$ ranged from 1/10 (volunteer 2) to 9/10 (volunteers 1 and 3). The mean percentage of dose recovered within 10 h was 33% (range 23–50%). There was a weak but significant negative correlation between urinary pH and amount of ephedrine in the urine; exercise did not consistently influence the urinary amount. These results illustrate the systemic availability of ephedrine upon intranasal administration and show that the therapeutic use of a nasal ephedrine formulation by an athlete on the day of a competition can lead to a urinary ephedrine concentration above 5 $\mu g\ mL^{-1}$, which is considered positive in current doping regulations of the International Union of Cyclists.

Ephedrine is present in many preparations for nasal application in rhinitis and sinusitis. In Flanders, doping regulations specify that persons participating in sport competitions should not use ephedrine. The International Olympic Committee and the International Union of Cyclists (UCI) also consider the use of ephedrine as doping. As for most other compounds, doping control for ephedrine in urine is mostly done in a qualitative way. When urine is found positive for ephedrine at the occasion of a doping control, sportsmen often maintain that they only applied a nasal preparation of ephedrine in therapeutic doses to relieve their rhinitis symptoms. Recently, the UCI introduced quantitative doping control for ephedrine, with the test only being considered positive if the urinary ephedrine concentration is higher than 5 $\mu g\ mL^{-1}$. It is not known whether the application of a nasal ephedrine preparation in therapeutic doses can lead to urinary ephedrine concentrations exceeding 5 $\mu g\ mL^{-1}$. The aim of the present study was to investigate the urinary excretion of ephedrine during repetitive application of a nasal ephedrine preparation. To examine whether exercise might influence the urinary excretion of ephedrine, the volunteers exercised on a bicycle ergometer during part of the experiment.

Materials and Methods

Subjects

Eight healthy male volunteers (21–30 years; weight 66–86 kg; height 1·71–1·90 m) gave written informed consent for the study, which was approved by the Ethics Committee of the Gent Medical School. Medical history, physical examination and routine laboratory tests revealed no clinically relevant abnormalities. None of the volunteers took medication on a regular basis; two were moderate smokers. Within one week before the experimental day (except for volunteer 2, where this was done 3 weeks before), the volunteers performed a maximal ergometer test on a bicycle ergometer to determine the $\dot{V}O_{2max}$ (20·0–43·8 mL min^{-1} kg^{-1}).

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Study design

After overnight fasting for 12 h with free access to water, the volunteers came to the experimental room. Urine was voided and at 0800 h the first nasal administration of ephedrine was performed; this was repeated 2, 4 and 6 h after the first application. The urine was collected for 10 h at hourly intervals. For 2 h after the last ephedrine administration, the volunteers exercised on a bicycle ergometer at a workload corresponding to 50% of their $\dot{V}O_{2max}$ (110–155 W). The exercise was interrupted after 1 h to allow the volunteers to void urine. The room temperature during exercise was 22–23°C.

The volunteers received a standard breakfast (including 300 mL of chocolate milk) immediately following the first administration and a standard lunch (including 250 mL of soup and 300 mL of water) immediately following the third administration. At 1, 2, 3, 5, 6, 7, 8 and 9 h after the first administration, they received 200 mL of water. Alcoholic beverages were not allowed from 12 h before the start of the study and during the study. Each administration of ephedrine consisted of 6 drops of a commercial 0·75% ephedrine solution (Endrine) applied in each nasal cavity. The drops were administered with the volunteers in supine position and the head in hyperextension; they remained in this position for about 2 min. Preliminary experiments showed that the weight of 6 drops of the 0·75% ephedrine preparation, obtained via the commercial pipette, was very reproducible. The mean of 9 weighings was $0·232 \pm 0·002$ g. Six drops thus correspond to 1·74 mg of ephedrine, which means that the total amount of ephedrine applied over the 4 applications was 13·92 mg.

Blood pressure and heart rate were measured before the

first administration of ephedrine, before, after one hour of and at the end of the exercise period, and at the end of the experimental session (10 h after the first application). The volume of the urine was measured and a urinary sample was stored under a liquid paraffin layer for determination of the pH within 24 h; another sample was stored at −20°C for determination of the ephedrine concentration.

Assays

Ephedrine in urine was determined by the HPLC method described by Brendel et al (1988) for the determination of pseudoephedrine in plasma and urine, with minor modifications for the conditioning of the BondElut extraction columns. Conditioning was performed with 1 mL of methanol, 1 mL of 0·3 M methanolic hydrochloric acid, 0·5 mL of a 60/40 (v/v) mixture of 0·03 M hydrochloric acid (pH 3·0) and acetonitrile followed by 1 mL of 0·3 M methanolic hydrochloric acid and 2 mL of water.

The chromatographic system consisted of a Waters 510 HPLC-pump and a Waters 484 variable wavelength monitor set at 205 nm. The column was a Lichrosorb 10-RP 18 (25 cm × 4·6 mm). The chromatograms were recorded on a Waters data and chromatography system with Baseline 810 chromatography software. The mobile phase consisted of 0·03 M sodium heptanesulphonate (adjusted to pH 3·0 with 0·3 M hydrochloric acid)—acetonitrile (70:30 v/v). The flow rate was set at 1·25 mL min^{-1}. The internal standard used was α-(methylaminomethyl)benzylalcohol (MAMBA).

Calibration lines were constructed by linear regression analysis of peak-area ratios of ephedrine to internal standard vs ephedrine concentration units. These calibration lines were prepared with urine samples containing between 0 and 5 μg mL^{-1} added ephedrine. The intra-assay coefficient of variation for 6 determinations of a urine pool containing 2·5 μg mL^{-1} ephedrine was 3·3%; the inter-assay coefficient of variation for 10 urine samples containing 2·5 μg mL^{-1} ephedrine was 5·8%. The accuracy was respectively 99·9 and 100·1%.

The urine pH was measured with an electrode (Portamess 751 Kalimatic, Knick, Germany).

Analysis of data

All results are given as mean ± s.e.m. Comparison of the results during exercise with those just before, and of the results after exercise with those during the second hour of exercise was by the signed-ranks test (Wilcoxon). Differences were considered significant for $P < 0.05$. For the relationship between pH and excretion, linear regression analysis was performed.

Results

The heart rate was 66 ± 4 beats min^{-1} before the first nasal application of ephedrine. Just before exercise it was 68 ± 3 beats min^{-1}, rising to 141 ± 5 and 153 ± 5 beats min^{-1} after 1 and 2 h of exercise, respectively. Two hours after exercise, it had returned to the pre-exercise values (72 ± 3 beats min^{-1}). Blood pressure readings at the same times were 125 ± 2/79 ± 2, 125 ± 5/75 ± 3, 152 ± 5/66 ± 3, 150 ± 4/68 ± 4, 117 ± 3/74 ± 2 mmHg.

The individual urinary concentrations and amounts are given in Table 1 and the mean results are shown in Fig. 1. In all volunteers, the urinary ephedrine concentration was higher than 5 μg mL^{-1} at one or more sampling points. On a total of 80 determinations, a urinary ephedrine concentration above 5 μg mL^{-1} was found 41 times. The number of urine samples with an ephedrine concentration exceeding 5 μg mL^{-1} ranged from 1/10 (volunteer 2) to 9/10 (volunteers 1 and 3). In all volunteers, the urinary ephedrine concentration clearly decreased during the 6th hour of the study but this corresponds to the large increase in urinary volume, probably induced by the liquid load at lunch. During

Table 1. Urinary excretion of ephedrine in 8 healthy volunteers after intranasal administration.

Hours	Volunteer 1	2	3	4	5	6	7	8	Mean ± s.e.m.
Urinary concn (μg mL^{-1})									
1	5·2	4·0	8·5	5·7	5·5	1·3	9·1	5·2	5·6 ± 0·9
2	6·9	4·6	5·5	4·4	3·6	3·3	9·4	4·4	5·3 ± 0·7
3	7·4	2·0	8·1	4·6	2·3	1·8	5·6	5·0	4·6 ± 0·9
4	8·2	2·7	8·4	3·6	3·9	1·3	4·2	5·3	4·7 ± 0·9
5	13·2	4·2	10·6	5·9	5·4	2·8	6·4	7·5	7·0 ± 1·2
6	3·1	1·2	4·2	1·0	1·2	0·9	1·6	0·9	1·8 ± 0·4
7	13·0	1·2	6·0	1·3	1·1	3·3	4·4	2·0	4·0 ± 1·4
8	9·3	2·0	7·9	4·1	1·5	9·5	11·4	3·0	6·1 ± 1·4
9	14·4	4·5	16·5	6·7	5·7	12·3	12·8	8·6	10·2 ± 1·6
10	10·7	12·1	16·0	5·5	5·4	9·4	6·5	4·1	8·7 ± 1·4
Urinary excretion rate (μg h^{-1})									
1	364·0	256·0	323·0	228·0	253·0	152·1	200·2	270·4	255·8 ± 23·6
2	414·0	280·6	302·5	189·2	183·6	188·1	263·2	237·6	257·4 ± 27·5
3	488·4	170·0	494·1	312·8	151·8	216·0	302·4	275·0	301·3 ± 46·3
4	467·4	226·8	579·6	417·6	249·6	247·0	281·4	349·8	352·4 ± 44·6
5	858·0	504·0	826·8	536·9	529·2	428·4	473·6	780·0	617·1 ± 61·5
6	620·0	414·0	630·0	365·0	414·0	360·0	344·0	378·0	440·6 ± 41·2
7	1001·0	330·0	750·0	344·5	396·0	561·0	396·0	340·0	514·8 ± 86·2
8	613·8	92·0	671·5	369·0	255·0	608·0	307·8	270·0	398·4 ± 73·8
9	777·6	652·5	1402·5	636·5	427·5	799·5	499·2	722·4	739·7 ± 105·1
10	791·8	907·5	1040·0	577·5	399·6	611·0	513·5	955·3	724·5 ± 81·9

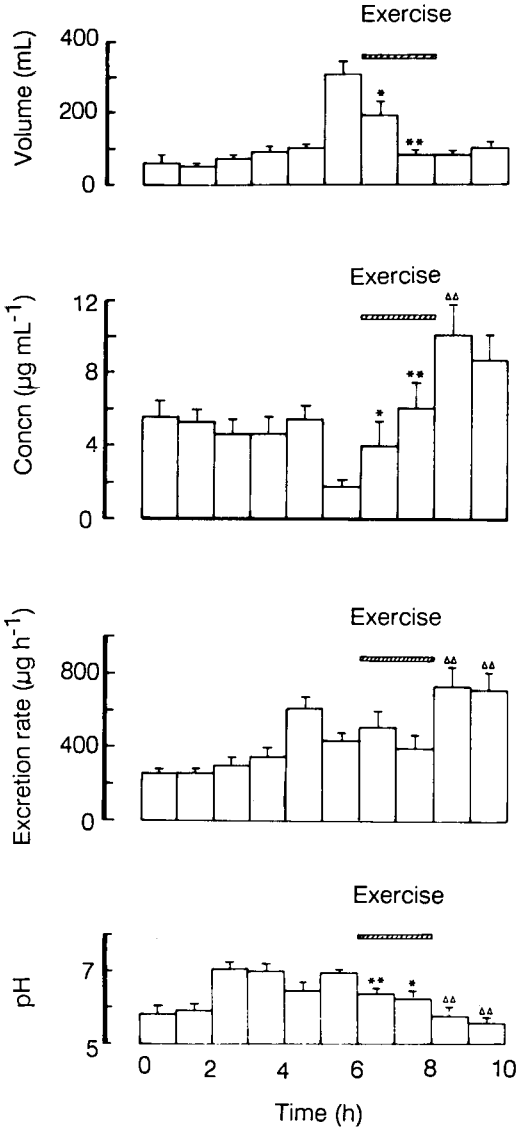

FIG. 1. Urinary volume, urinary ephedrine concentration and excretion rate, and urinary pH in 8 healthy volunteers. Ephedrine was administered intranasally at 0, 2, 4 and 6 h. Exercise was performed on a bicycle ergometer. Means ± s.e.m. are shown. $*P<0.05$, $**P<0.01$ compared with the value just before exercise. $\Delta\Delta P<0.01$ compared with the value during the second hour of exercise.

exercise, excretion did not change but during the hour after exercise, concentration and amount increased in all subjects.

The mean urinary pH ranged between 5·57 ± 0·15 and 7·06 ± 0·16; for all samples, the urinary pH range was 5·03–7·75. The maximal pH difference between samples within one volunteer ranged from 1·19 (volunteer 1) to 2·45 (volunteer 2). Urinary pH significantly decreased during exercise; a further decrease was observed in the 2 h after exercise. There was a weak but significant correlation ($r = -0.38$; $P<0.001$) between urinary pH and the amount of urinary ephedrine. The mean excretion over the 10 h of observation was 4·60 ± 0·48 mg, corresponding to 33% of the dose applied in the nasal cavities (range 23 (volunteer 5) to 50 (volunteer 3) %).

Discussion

The aim of this study was to investigate whether urinary ephedrine concentrations above 5 μg mL^{-1} were reached upon intranasal administration of therapeutic doses. A commercial 0·75% ephedrine solution for nasal application (drops) was used and the maximal dose mentioned in the Belgian package insert was applied. The application was well supported and none of the volunteers reported side-effects.

The most frequent reason for intranasal application of drugs is still the treatment of local diseases. Yet, the observation of systemic side effects upon intranasal dosing suggested that systemic availability of some drugs can be obtained via the nasal mucosa (Parr 1983); the subject was recently reviewed (Pontiroli et al 1989). For drugs such as propranolol and neostigmine which show variable absorption after oral administration, the intranasal route is superior to the oral route (Hussain et al 1980; Broggini et al 1991). Ephedrine is a basic drug that is well absorbed upon oral administration (Wilkinson & Beckett 1968; Welling et al 1971; Sever et al 1975); it is, however, also used for local treatment of rhinitis. Our results clearly illustrate that ephedrine is systemically available upon intranasal administration. Although the 10 h observation period was limited and not aimed at studying the complete urinary recovery upon intranasal administration, the mean percentage of the intranasal dose recovered within the period was 33%. For comparison, upon single oral dosing of 25 mg ephedrine, the percentage excreted in 48 h varied from 70 to 80% (Welling et al 1971). After nasal application of ephedrine, nasal absorption thus occurs but part of the ephedrine probably reaches the systemic circulation via intestinal absorption; the clearance of nonabsorbed material from the nasal cavity to the pharynx and thus to the stomach is well documented (Hardy et al 1985). The urinary ephedrine concentration often exceeded 5 μg mL^{-1}. This means that the normal use of a nasal ephedrine formulation on the day of a competition can lead to a positive doping control.

It has been clearly established that urinary pH influences the excretion of ephedrine; alkalinization of the urine decreases the urinary excretion because of the increased passive reabsorption of non-ionized drug in the renal tubules (Wilkinson & Beckett 1968). In our study, there was a weak though significant negative correlation between urinary pH and urinary amount excreted. Welling et al (1971) found no significant correlation between the ephedrine excretion rate and the urinary pH in healthy volunteers. The pH of most of the samples in their study fell within the pH range 5·32–7·28, and they suggested that the changes in percentage of ionized drug within this pH range are too small to observe significant correlations.

Exercise can influence the pharmacokinetics of drugs by changes in absorption, distribution and elimination (Somani et al 1990; Van Baak 1990). During physical activity, lactic acid is produced leading to a decrease in blood pH (Dal Monte & Dragan 1988) and the urinary pH may decrease during exercise (Ylitalo et al 1977). We also observed a decrease in urinary pH during exercise and this would increase the urinary ephedrine excretion. On the other hand, renal plasma flow decreases in an intensity-dependent way during exercise (Castenfors 1977) and literature data show

that the renal clearance of some drugs is reduced during exercise (Van Baak 1990). The degree of heart-rate increase obtained during exercise in our study shows that a clear reduction of renal blood flow must have occurred (see Castenfors 1977), which might reduce the ephedrine excretion. No significant influence of exercise on the urinary excretion of ephedrine was observed in our study. A significant increase was observed in the hours after exercise, which is difficult to explain.

In conclusion, the use of a nasal ephedrine formulation in normal doses can lead to urinary concentrations of ephedrine above 5 $\mu g\ mL^{-1}$.

Acknowledgements

R. A. Lefebvre is a Senior Research Associate of the National Fund for Scientific Research.

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