Brit. J. Pharmacol. (1966), **26**, 468–472.

INFLUENCE OF URINARY *p*H ON THE DEGREE AND DURATION OF ACTION OF AMPHETAMINE ON THE CRITICAL FLICKER FUSION FREQUENCY IN MAN

BY

J. V. SMART

From the Department of Statistics, Research and Development, Smith, Kline & French Laboratories, Welwyn Garden City, Herts.

AND

P. TURNER*

From Clinical Pharmacology, Medical Professorial Unit, St. Bartholomew's Hospital, London, E.C.1

(*Received November* 24, 1965)

The rate of excretion of amphetamine in man is very dependent on urinary *p*H (Beckett, Rowland & Turner, 1965; Asatoor, Galman, Johnson & Milne, 1965). A low *p*H results in the rapid elimination of the drug, whereas an alkaline urine delays its excretion. Retention of a drug within the body does not necessarily imply prolonged action, however, and it seemed desirable, therefore, to determine if the rate of excretion of amphetamine as influenced by urine *p*H modified the extent and duration of action of the drug upon the central nervous system. Measurement of critical flicker fusion frequency is a sensitive test for assessing centrally acting drugs (Roback, Krasno & Ivy, 1952; Holland & Gooch, 1962; Ideström & Cadenius, 1963; Turner, 1965a, b). Dexamphetamine sulphate (10 mg) did not produce significant changes in this test (Turner, 1965a) although it abolished the depression of critical flicker fusion frequency produced by antihistamine drugs (Roback *et al.*, 1952) and by amylobarbitone sodium (Turner, 1965b). Dr Hannah Steinberg (personal communication) suggested that (±)-amphetamine sulphate might be effective, however, and this paper describes the results of experiments investigating the effect of this drug upon critical flicker fusion frequency, and the modification of its effect by varying urine *p*H. The experimental design also permitted an evaluation of the effect of amphetamine sulphate on the modification of critical flicker fusion frequency by adaptation to intermittent light of varying frequency described by Alpern & Sugiyama (1961) and confirmed by Turner (1964).

METHODS

The technique used was that of Turner (1964), the essentials of which are as follows. Subjects were seated in the middle of a room 12 × 10 ft, with grey walls and ceiling, using daylight illumination. The eyes and the flickering light sources were in the same horizontal plane separated

* Author for correspondence and reprint requests.

by 60 cm. The source was a neon lamp of luminance 350 lumens/m^2 exposing an area of 0.64 cm^2, driven by a rectangular pulse generator (Solatron Pulse Generator Type GO 1101.2). The lamp was viewed against the background of a brown sheet of hardboard (luminance 10 lumens/m^2) behind which its socket was mounted. The experimenter sat opposite the subject with the apparatus between them, and he adjusted the flicker rate in 0.5 cycles/sec steps. The subject closed his eyes during changes in flicker frequency. On opening them, he was instructed, without a limit on time, to look directly at the light and decide if it appeared to flicker or not. The critical flicker fusion frequency was taken to be the fastest rate at which the source was considered to be flickering as opposed to being steady. This threshold was determined either by an approach from a lower to a higher frequency (ascending threshold) or *vice versa* (descending threshold).

The light source flickering at either 20 or 50 cycles/sec for 1 min provided the adapting stimulus, after which ascending or descending thresholds were measured. To eliminate order effects ascending and descending thresholds after exposure to 20 and 50 cycles/sec were determined randomly using a latin-square sequence.

An acid urine *p*H of 5.0 or less was produced and maintained by the administration of ammonium chloride tablets, and an alkaline *p*H of 8.0 or more by giving sodium bicarbonate in solution by mouth. Medication was started in the evening before the test. The *p*H was checked during the experiment with a Cambridge bench *p*H meter.

Results were submitted to analyses of variance and considered statistically significant if their *P* values were equal to or less than 0.05.

RESULTS

Determination of the effect of amphetamine sulphate on critical flicker fusion frequency

Four healthy subjects aged between 22 and 30 years were studied. At 0900 hr, 1.5 hr after a light breakfast, four measurements of critical flicker fusion frequency were made, namely ascending and descending thresholds after exposure to 20 and 50 cycles/sec with 2 min between each measurement. Amphetamine sulphate (15 mg) or a placebo tablet identical in appearance to the active preparation was then given. The four thresholds were next measured at 2 and 4 hr after administration. Each subject received both amphetamine and placebo in random order at an interval of 2 to 4 days. From waking and until the termination of the threshold measurements they abstained from " stimulants " such as tea, coffee, and nicotine. Neither they nor the observer were aware of the identity of the tablet administered. No control of urine *p*H was exercised in this experiment.

The effects of drug and placebo on mean critical flicker fusion frequency are expressed graphically in Fig. 1. They confirm our previous observations (Turner, 1965a) that a placebo is associated with a fall in critical flicker fusion frequency in the first 4 hr after administration, but that this does not occur after amphetamine sulphate, a significant difference between them being seen at 4 hr. Analysis of variance shows that there is a significant difference between mean thresholds after exposure to 20 and 50 cycles/sec, both before and after administration of both drug and placebo, and that no significant interaction was found. In the absence of such an interaction, tables of means are not included.

Investigation of the effect of modification of urine pH on action of amphetamine

Six healthy subjects aged between 22 and 35 years were studied. As before, four measurements were made before and at 2.5, 5 and 8 hr after administration of amphetamine sulphate (15 mg) and placebo under double-blind conditions. Each

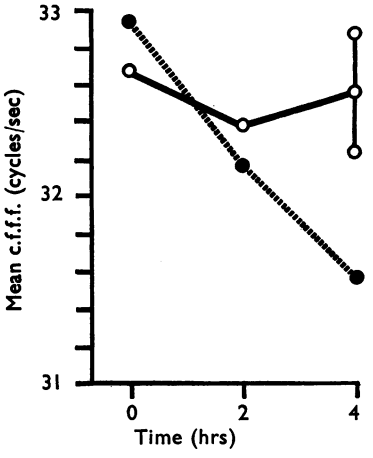

Fig. 1. Changes in critical flicker fusion frequency (c.f.f.f., means of ascending and descending thresholds after exposure to 20 and 50 cycles/sec) after administration of 15 mg of amphetamine sulphate (solid line) and placebo (broken line). 95% confidence limits were all of the same order and are shown only for amphetamine sulphate at 4 hr (n = 4).

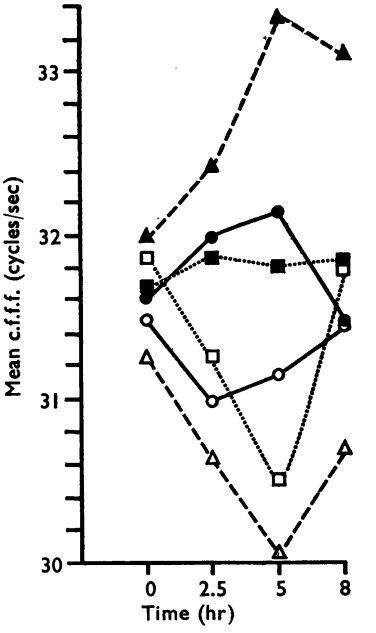

Fig. 2. Changes in critical flicker fusion frequency (c.f.f.f., means of ascending and descending thresholds after exposure to 20 and 50 cycles/sec) after administration of amphetamine sulphate (solid symbols) and placebo (open symbols) under conditions of urine acidity (□——□), alkalinity (△——△) and uncontrolled *p*H (○——○) (n = 6).

preparation was given on three separate occasions under conditions of urine acidity, alkalinity, and without control.

The analysis of variance (Table 1) shows a significant difference between drugs (amphetamine *versus* placebo). *p*H alone did not have a significant effect, but the interaction drugs *versus* *p*H was highly significant (Table 2). These results are illustrated in Fig. 2 which shows that while mean critical flicker fusion frequency fell during the first 5 hr after taking the placebo, it rose after administration of amphetamine, and that an alkaline urine produced a significantly greater increase in critical flicker fusion frequency after amphetamine than did an acid urine. This increase was in the duration as well as in the magnitude of the effect.

TABLE 1

ANALYSIS OF VARIANCE SHOWING EFFECTS OF DIFFERENCES BETWEEN DRUGS (AMPHETAMINE VERSUS PLACEBO), AND URINE *p*H, WITH THEIR INTERACTION

Variant	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Drugs	1	148·03	148·03	19·202	<0·01
*p*H	2	2·22	1·11	—	—
Drugs *v.* *p*H	2	80·02	40·01	5·190	0·025–0·01
Error	20	154·18	7·709		

TABLE 2

CRITICAL FLICKER FUSION FREQUENCIES TO SHOW DRUGS AGAINST URINE *p*H INTERACTION

Each value is the mean (summed over subjects and times) of ninety-six measurements. 5% critical difference =0·84 cycles/sec

	Critical flicker fusion frequency (cycles/sec) for urine		
	Acid	Uncontrolled	Alkaline
Placebo	31·35	31·27	30·65
Amphetamine	31·79	31·81	32·72

DISCUSSION

These results demonstrate that amphetamine sulphate (15 mg) produces a significant rise in critical flicker fusion frequency, and that this effect may be increased and prolonged by the simultaneous production of an alkaline urine. Variation of urine *p*H alone had no significant effect on critical flicker fusion frequency. This is in accord with the findings of Beckett *et al.* (1965) and Asatoor *et al.* (1965) that an acid *p*H increases the excretion of dexamphetamine sulphate while an alkaline urine results in its retention. These results may be explained by the theory of non-ionic diffusion of basis in the renal tubules (Milne, Schribner & Crawford, 1958). As urine *p*H falls, the ratio of un-ionized to ionized amphetamine in the intraluminar fluid of the tubules falls relative to that in the plasma. Less drug is, therefore, reabsorbed, and its excretion rate is increased.

It appears, therefore, that the central stimulant action of amphetamine may be prolonged and increased under conditions of urine alkalinity, and that caution must be exercised in administering conventional doses, especially in the form of prolonged-action capsules to patients whose urine is alkaline.

It is of interest that amphetamine sulphate produced a rise in critical flicker fusion frequency whereas dexamphetamine sulphate did not (Turner, 1965a), although it prevented depression by amylobarbitone sodium (Turner, 1965b). The reason for this is obscure, but may be related to specificity of the central receptors concerned.

The adaptation of critical flicker fusion frequency to light flickering at different frequencies (Alpern & Sugiyama, 1961 ; Turner, 1964, 1965c) is stable and unaffected by physiological procedures such as hyperventilation and rebreathing, and by amylobarbitone sodium (60 and 120 mg), chlorpromazine (10 and 25 mg) and phenmetrazine theoclate (30 mg) (Turner, 1965a). These results demonstrate that amphetamine sulphate (15 mg) also fails to influence this phenomenon.

The fall in critical flicker fusion frequency following placebo which was seen in both experiments has been observed repeatedly (Turner, 1965a,b) although other authors have not described it. It may be due to factors in the method used such as uncontrolled pupil size, although in other experiments (Turner & Patterson, unpublished) no significant pupillary changes were demonstrated. Whatever may be the cause for this depression, it is abolished and reversed by amphetamine sulphate.

It appears that the measurement of critical flicker fusion frequency is a reliable and sensitive index of the effects of drugs upon the central nervous system, permitting a study both of their degree and duration of action, under varying physiological and pathological conditions.

SUMMARY

1. Oral amphetamine sulphate (15 mg) significantly elevated the critical flicker fusion frequency in normal subjects, or prevented the fall in threshold associated with a placebo.

2. Under conditions of urine alkalinity (pH>8.0) the magnitude and duration of elevation of critical flicker fusion frequency by amphetamine sulphate (15 mg) was significantly greater than under acid conditions (pH<5.0).

3. Variations in urine pH did not significantly influence critical flicker fusion frequency following administration of a placebo.

4. Amphetamine sulphate did not influence the modification of critical flicker fusion frequency by intermittent light of varying frequency.

One of us (P.T.) is in receipt of a Wellcome Senior Research Fellowship in Clinical Science. Valuable assistance was given by D. S. Patterson.

REFERENCES

ALPERN, M. & SUGIYAMA, S. (1961). Photic driving of the critical flicker freguency. *J. opt. Soc. Amer.* **51**, 1379–1385.

ASATOOR, A. M., GALMAN, B. R., JOHNSON, J. R. & MILNE, M. D. (1965). The excretion of dexamphetamine and its derivatives. *Brit. J. Pharmacol.*, **24**, 293–300.

BECKETT, A. H., ROWLAND, M. & TURNER, P. (1965). Influence of urinary pH on excretion of amphetamine. *Lancet*, i, 303.

HOLLAND, H. C. & GOOCH, R. N. (1962). Critical flicker threshold as a function of the concentration of nitrous oxide. *Nature (Lond.)*, **193**, 738–739.

IDESTRÖM, C. M. & CADENIUS, B. (1963). Chlordiazepoxide, dipiperon and amobarbital. Dose effect studies in human beings. *Psychopharmacolgia (Basel)*, **4**, 235–246.

MILNE, M. D., SCHRIBNER, B. H. & CRAWFORD, M. A. (1958). Non-ionic diffusion and the excretion of weak acids and bases. *Amer. J. Med.*, **24**, 709–729.

ROBACK, G. S., KRASNO, L. R. & IVY, A. C. (1952). Effect of analeptic drugs on the somnifacient effect of seconal and antihistamines as measured by the flicker fusion threshold. *J. appl. Physiol.*, **4**, 566–574.

TURNER, P. (1964). Critical flicker fusion frequency and its modification by a conditioning stimulus of flickering light. *J. Physiol. (Lond.)*, **171**, 6–8*P*.

TURNER, P. (1965a). *The Changes in Critical Flicker Fusion Frequency Induced by Some Physiological Procedures and by Drugs, Together with Observations on the Reliability of Olfactory Testing.* M.D. thesis at the University of London.

TURNER, P. (1965b). Effect of a mixture of dexamphetamine and amylobarbitone on critical flicker fusion frequency. *J. Pharm. Pharmacol.*, **17**, 388–389.

TURNER, P. (1965c). The modification of critical flicker fusion frequency by an adapting stimulus of flickering light. *Vision Res.*, **5**, 463–470.