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Cardiovascular effects of THIP in the rat

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γ-Aminobutyric acid (GABA) agonists decrease blood pressure (BP) and heart rate (HR) in most species (for ref. see Persson 1980a). The most potent and well recognized GABA agonist is muscimol (Curtis et al 1971; Johnston 1976). However, discrepant results following systemic and intracerebroventricular (i.c.v.) administration of muscimol on, e.g. BP (Persson 1980a; Persson & Henning 1980) and prolactin secretion (Müller et al 1979), as well as poor anticonvulsant effects after systemically administered muscimol (Meldrum 1979) have raised questions as to whether the rapid metabolism of muscimol in the liver (Baraldi et al 1978; Maggi & Enna 1978) prevents sufficient peripherally administered muscimol to reach the brain unchanged to subsequently activate GABA receptors, or whether the observed effects are due to a derivative.

Tetrahydroisoxazolpyridine (THIP) is a stable muscimol analogue which passes the blood brain barrier easily and has effects qualitatively similar to those of muscimol (Scheel-Krüger et al 1979). Since THIP is not rapidly metabolized, and consequently passes unchanged into the brain, the BP and HR effects of i.p. and i.c.v. administered THIP have been compared with those of muscimol (see above) to see if this would indirectly answer the question whether the discrepant response to systemically administered muscimol is due to rapid peripheral inactivation.

Method

Male Sprague-Dawley rats (Anticimex, 220–250 g) were used. Mean arterial BP and HR were recorded in conscious unrestrained or anaesthetized rats through indwelling catheters (Portex Tubing PP50, carotid artery) connected to Statham P23Dc transducers writing on a Grass Polygraph (Trolin 1975). Intravenous catheters were implanted into the jugular vein as described by Trolin (1975) and i.c.v. catheters (lat. ventricles) as described by Garcia-Sevilla et al (1978). Drugs used were: picrotoxin HCl, THIP, bicuculline HCl and clonidine HCl. The drugs were given i.v. at a volume of 2 ml kg^{-1}, i.p. at a volume of 10 ml kg^{-1} and i.c.v. at a volume of 20 μl/rat.

Results and discussion

Intraperitoneally administered THIP in moderate to toxic doses to conscious rats had negligible cardiovascular effects (1–20 mg kg^{-1}, n = 10, data not shown). Intracerebroventricular administration of THIP in the 4–10 μg dose range reduced BP and HR (Fig. 1). These effects, including the hypertension to the lowest dose of THIP employed (2 μg), were probably due to activation of GABA receptors since they were qualitatively similar to those of GABA and muscimol (Persson 1980b) and since they were potentiated by barbiturates which is typical of GABA-mediated events (Lodge & Curtis 1978). Furthermore, the HR reduction to THIP (10 μg i.c.v.) was antagonized by i.v. administration of the GABA antagonist bicuculline (Krnjevic 1974) (HR rose from 337 ± 12 to 409 ± 16 beats min^{-1}, n = 8, original base line was 411 ± 8 beats min^{-1}). This antagonism was probably specific since the same dose of bicuculline (0·5 mg kg^{-1} i.v.) did not antagonize the centrally induced bradycardia following i.v. administration of clonidine (7 μg kg^{-1}); HR was actually further decreased (from 359 ± 18 to 295 ± 23 beats min^{-1}, n = 6, original base line was 409 ± 12 beats min^{-1}). In these interaction studies the animals were slightly anaesthetized (chloral hydrate) since clonidine has slight effects in conscious normotensive animals (Trolin 1975).

The interaction between THIP and the GABA antagonist picrotoxin (Krnjevic 1974) was also studied but the results from that experiment at first sight seem confusing, i.e. pretreatment with picrotoxin did not prevent the BP and HR reduction to THIP. However, administration of picrotoxin by itself raised BP and reduced HR (see Fig. legend) and the results could also be interpreted as THIP antagonizing the cardiovascular effects of picrotoxin. These results demonstrate that because of the ubiquity of GABA mechanisms in both excitatory and depressive cardiovascular centres in the brain, the net result following a general activation of GABA receptors by drugs is hard to predict. Probably the general state of activity of sensitive structures, pharmacokinetic phenomena when drugs are given by different routes of administration and the possibility of different GABA receptors are factors of importance. Thus, it seems that GABA antagonists (DiMicco et al 1977) and GABA agonists (Persson 1980b) have biphasic responses, possibly due to the fact that interference of GABA mechanisms has diverging cardiovascular effects in e.g. the forebrain and the brain stem (DiMicco et al 1979). In light of this it is more understandable why effects on HR and BP after i.c.v. THIP were not necessarily parallel and administration of THIP after picrotoxin pretreatment actually increased HR. A possible locus of the latter interaction has been defined by DiMicco et al (1979) who

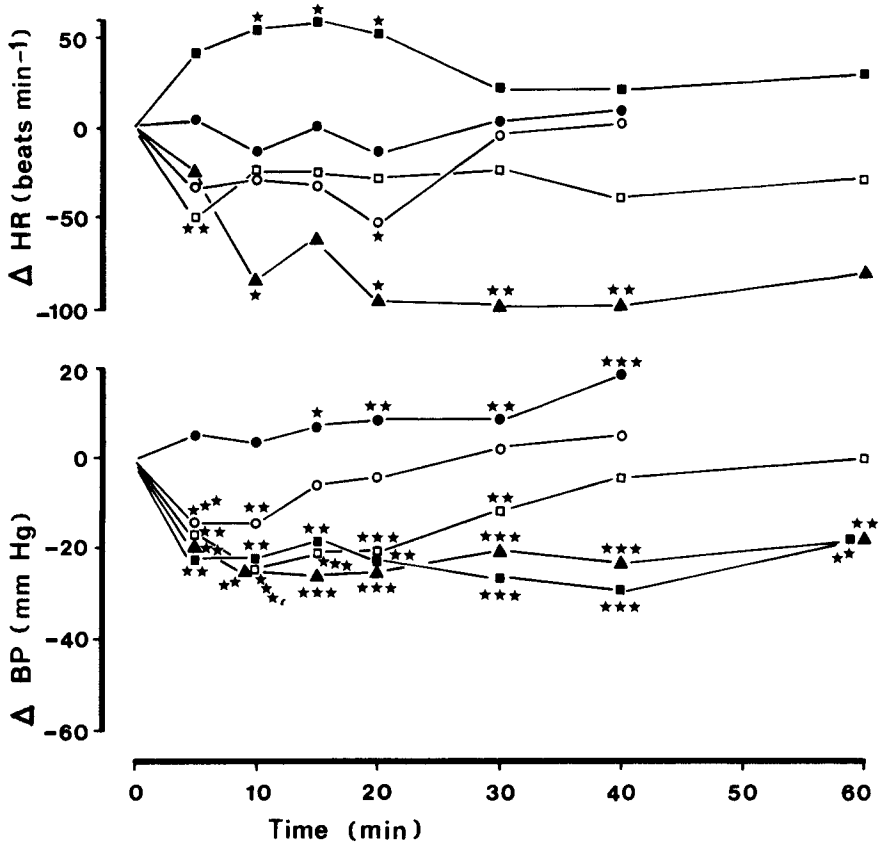

FIG. 1. Cardiovascular effects of intracerebroventricular administration of THIP. The values are means of arterial blood pressure (BP mm Hg, lower ordinate) and heart rate (HR beats min^{-1}, upper ordinate) expressed as changes from basal levels (time 0). Abscissa: time (min). Basal levels (noted after various pretreatments) are indicated within brackets as: BP mm Hg/HR beats min^{-1}. ● (2 μg, n = 3, 113 ± 6/420 ± 20), ○ (4 μg, n = 7, 107 ± 6/389 ± 15), □ (10 μg, n = 8, 112 ± 5/381 ± 18), ▲ (10 μg, 20 min after i.p. chloral hydrate 140 mg kg^{-1}, n = 4, 95 ± 5/399 ± 55), ■ 10 μg, 20 min after i.p. picrotoxin 2 mg kg^{-1}, n = 6, 134 ± 6/280 ± 24). Note that pretreatment with chloral hydrate reduced BP from original base line values (110 ± 4/388 ± 20) and picrotoxin raised BP and reduced heart rate from original base line values (121 ± 4/340 ± 12). Asterisks indicate significances of differences from own base line at time 0 (analysis of variance followed by *t*-test on absolute values). $*P < 0{\cdot}05$, $**P < 0{\cdot}025$, $***P < 0{\cdot}005$.

demonstrated a specific GABA input to the n. ambiguus, involved in HR regulation.

The lowest dose of THIP employed (2 μg) increased BP, as has prevously been demonstrated for low doses of muscimol (Persson 1980b). As discussed above this could possibly indicate the existence of different GABA receptors. However, the delay of the hypertensive response (compared with the fall occurring after 4–10 μg) would suggest that the BP rise could be due to indirect effects or due to pharmacokinetic phenomena, i.e. the low dose of THIP causing a BP rise does not penetrate to the site(s) at which activation of GABA receptors has hypotensive effects.

In conclusion, i.c.v. administered THIP had significant cardiovascular effects, probably due to activation of GABA receptors, but systemically administered THIP was largely ineffectual in this respect. Thus, THIP had cardiovascular effects similar to those of muscimol. This implies that the disparate effects of muscimol given i.c.v. or systemically cannot automatically be ascribed to rapid metabolism before its entering into the brain. Rather, the results support the contention that GABA receptors are ubiquitous and that pharmacokinetic events determine the overall pharmacological effects.

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Effect of chlorpheniramine, promethazine and cimetidine on human sperm motility in-vitro

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Sperm motility is affected by some centrally active drugs, phosphodiesterase inhibitors and drugs with local anaesthetic (or membrane stabilizing) activity (Amelar et al 1980; Hong et al 1981a). We have investigated the effects of some H_1 and H_2 antihistamines on human sperm motility.

Method

Fresh human semen samples collected from healthy volunteers who were non-smokers and non-alcoholics, and patients attending an infertility clinic were used within 2 h of collection. Only samples with a sperm count higher than 15×10^6 ml^{-1} and a transmembrane migration ratio (TMMR)—which is the % of progressive forward moving sperms—higher than 20%, were used (Hong et al 1981b). All drugs were dissolved in phosphate saline at pH 7·3 (Dulbecco A Oxoid). Sperm motility was measured using the ability of forward moving sperms to move across the 5 μm pores of a Nucleopore membrane during a 2 h incubation at 37 °C. The motility of sperms in semen buffer mixture was used as a control and those of semen-drug mixtures were expressed as percentages of the control. Aliquots of semen were mixed with buffer or drug in the ratio 2 : 1. For each of the drug dose response curves, 6 samples were tested. When histamine effects on promotheazine-induced changes in sperm motility were studied, the volumes of each of these drugs were halved so that the ratio of semen to drug mixture was kept a constant at 2 : 1.

Drugs tested in this experiment included histamine phosphate (BDH), chlorpheniramine maleate (Glaxo), promethazine hydrochloride (May & Baker) and cimetidine (SKF). The concentrations of drugs that decreased sperm motility to 50% of control (ED50) were obtained from semi-logarithmic concentration-effect curves. Statistical analysis was carried out using a paired 2-tailed *t*-test.

Results

Table 1 shows the effect of histamine and histamine antagonists on sperm motility. Neither histamine nor cimetidine in concentrations between 1 and 10 mM produced significant change in sperm motility. The two classical antihistamines, promethazine and chlorpheniramine, both produced a dose-dependent decrease in sperm motility, promethazine being the more potent. The ED50 values for promethazine and chlorpheniramine were 2·5 and 7·5 mM respectively. Table 2 shows the effect of histamine on promethazine-induced reduction of sperm mobility. Histamine antagonized promethazine-induced inhibition of sperm motility, the inhibitory effect being reduced significantly ($P < 0.01$) as the concentration of histamine was increased.

Table 1. Drug effects on the transmembrane migration ratio of human sperms (expressed as % control motility) (n = 6).

Drug concn (mM)	Histamine (mean ± s.d.)	Cimetidine (mean ± s.d.)	Prometh-azine (mean ± s.d.)	Chlorphenir-amine (mean ± s.d.)
1·0	101·0 ± 4·00	100·6 ± 3·2	*91·8 ± 2·71	94·3 ± 3·07
2·5	104·0 ± 6·07	98·4 ± 2·4	*53·2 ± 3·37	*70·0 ± 6·93
5·0	106·5 ± 7·26	98·2 ± 3·1	*38·0 ± 2·45	*58·2 ± 5·94
7·5	101·8 ± 5·31	96·0 ± 2·8	*22·2 ± 4·00	*47·8 ± 3·31
10·0	99·7 ± 4·27	89·8 ± 2·6	*15·2 ± 3·47	*39·8 ± 2·84

* Compared with histamine $P < 0.01$.

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