Biphasic effect of L-5-HTP in the Vogel conflict model

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Abstract. The effect of L-5-HTP (25–400 mg/kg IP) following inhibition of the peripheral aromatic amino acid decarboxylase by means of benserazide (25 mg/kg IP) was investigated in a test modified from Vogel's drinking conflict model. At 50 mg/kg an anti-conflict action was detected, while higher doses (100–400 mg/kg) decreased punished responding. A lower dose (25 mg/kg) had no effect. Non-specific effects – such as alterations in muscle tone, in motivation to drink or in the sensitivity to electrical shock – could not explain the anxiolytic- and anxiogenic-like actions of 50 and 100 mg/kg, respectively. The bi-phasic effect of L-5-HTP is discussed in terms of different subpopulations of central serotoninergic receptors, possibly exerting opposing influences on conflict responding. The study emphasises the importance of 5-HT mechanisms in anxiety, and the possibility of finding novel anxiolytics among drugs selectively affecting central 5-HT neurotransmission.

Key words: 5-HT – L-5-HTP – Anxiolytic – Anxiogenic – Drinking – Conflict – Vogel test – Rat

Brain serotonin (5-HT) systems are frequently implicated in the actions of agents exerting anxiolytic-like effects (for references see, e.g. Iversen (1985). The 5-HT involvement in the anxiety-reducing effects of benzodiazepines is a matter of controversy (cf, e.g. Tye et al. 1977; Kilts et al. 1982). However, it is worth noting that some novel non-benzodiazepine, putatively anxiolytic, agents (e.g. buspirone, ritalserin and TVX Q 7821 = isapirone) have prominent serotonin-mimetic or -lytic properties (Hjorth and Carlsson 1982; Leysen et al. 1985; Traber et al. 1984). Indeed, it has been suggested that the anxiolytic effects of these compounds may result from their abilities to interact with central 5-HT sites (e.g. Ceulemans et al. 1985; Peroutka 1985).

Moreover, we have recently demonstrated that the putative 5-HT (5-HT1A; Middlemiss and Fozard 1983) receptor agonist 8-OH-DPAT (Arvidsson et al. 1981; Hjorth et al. 1982), exerts anticonflict actions in naive rats in a modified Vogel conflict test procedure (Engel et al. 1984). In contrast, after para-chlorophenylalanine (PCPA) pretreatment only “pro”-conflict effects of this agent and of L-5-HTP were obtained.

Given this background and a recent clinical report on anxiolytic effects of L-5-HTP (Kahn and Westenberg 1985), it was deemed of interest to further investigate the actions of the 5-HT precursor in the aforementioned animal experimental model – considered to detect potential anxiolytic-like drug activity (Vogel et al. 1971).

Materials and methods

Subjects. Male rats of the Sprague-Dawley strain (ALAB, Sollentuna, Sweden), weighing 180–250 g, were used in the experiments. The animals were kept under controlled standardised environmental conditions (temperature 25 °C; humidity 60%; lights on 5.30 a.m.–5.30 p.m.) for at least 1 week after arrival in the Department until used in the experiments. Laboratory chow (R3; Astra-Ewos, Södertälje, Sweden) and tap water were freely available prior to commencement of the experimental procedures.

Drugs. L-5-Hydroxytryptophan (L-5-HTP; Sigma, St. Louis, MO, USA) was dissolved in a minimal quantity of 1 N HCl and made up to volume with 0.9% NaCl under gentle warming, after which pH was adjusted. Benserazide (RO 4-4602/1; generously supplied by Hoffmann-La Roche, Basle, Switzerland) was dissolved in 0.9% NaCl. Drug and vehicle injections were given IP, in a volume of 2 ml/kg.

Conflict testing. Conflict testing was carried out as previously described (Engel et al. 1984; Hjorth et al. 1986). On the 1st day of the experiment, the rats were adapted for 5 min to the test chamber. This was a Plexiglas box (inner dimensions 30 x 24 x 20 cm) enclosed in a sound-proof cage, and equipped with a grid floor of stainless steel bars and a drinking bottle containing a 5.5% (w/v) glucose solution. An electric shock (0.16 mA; delivered for 2 s every other second by means of a commercially available shock generator: Grason Stadler E1064) could be applied between the spout of the drinking bottle and the grid floor. The shock intensity (0.16 mA) was chosen on the basis of previous experiments, establishing a level of responding (drinking attempts) in control animals that would allow the detection of potential anti- as well as “pro”-conflict drug actions, i.e. increases and decreases, respectively, in the number of shocks accepted during the session.

After the initial adaptation the animals were water deprived for 24 h and then placed in the test chamber for a further 5 min adaptation, during which they had free access to the drinking bottle. They were then allowed a 30-min
After another 24 h water deprivation period, the rats were (following drug or vehicle treatment) again placed in the test chamber and allowed to drink the glucose solution for 30 s. Immediately thereafter every subsequent drinking attempt was punished with an electric shock. The number of shocks accepted during a 10-min experimental session was recorded. The anti-conflict effect of diazepam and its reversal by the benzodiazepine receptor antagonist RO 15-1788 has previously been demonstrated using this test paradigm (Liljequist and Engel 1984). To minimise diurnal variations in behavior all experiments were carried out between noon and 3.00 p.m. The animals were used only once in these studies.

**Sensitivity to electrical shock.** Shock sensitivity was likewise assessed in the conflict testing chamber, but in a separate group of animals and with the electrical shock delivered through the grid floor instead of the drinking spout. The conflict testing chamber and the shock generator regulating device were situated in separate rooms. The shock intensity was manually raised step-wise by one assistant in the ‘‘shock-generator room’’, from 0.05 mA (steps: 0.05, 0.06, 0.08, 0.10, 0.13, 0.16, 0.20, 0.25, 0.30, 0.40 mA; shock duration: 2 s; inter-shock interval: 10 s) until a reaction indicative of perceived shock (jerk/jump or similar) was observed by a second assistant in the ‘‘conflict-testing room’’. The shock reaction occurred typically between 0.13 and 0.16 mA in vehicle-treated rats. The observer was blind to the treatment of the animals and to the current intensity applied. The handling procedure for the animals used in these experiments was identical to that described above for conflict testing, including water deprivation and prior adaptation to the test chamber.

**Drinking motivation.** Motivation to drink was estimated in another group of animals. For these measurements the rats were deprived of water using a schedule and handling procedure identical to that applied for the conflict test (cf above); i.e. water-deprivation for 24 h, a free 30-min drinking session in the ‘‘home cages’’, followed by water-deprivation for another 24-h period. Subsequent to drug or vehicle treatment they were placed into individual ‘‘home’’ cages with free access to 5.5% glucose solution. The amount of liquid drunk during a 10-min period was determined.

**Motor ability.** A treadmill (Rotarod) with a diameter of 6 cm and a rotational speed of 8 rpm was used to assess the possibility that L-5-HTP negatively affected muscular tone or motor co-ordination. The animals were trained daily to walk on the treadmill in 5-min sessions. A total of three to four training sessions was typically required until they were able to pursue the treadmill walking for 300 consecutive seconds without falling off. Animals that failed to fulfil this criterion in two subsequent training sessions were excluded from the experiments (usually one or two out of ten rats). The test was performed 24–36 h after the last training session. Following drug or vehicle treatment, the amount of time (s) spent walking on the treadmill before falling off, during the 300-s test session was recorded.

**Statistics.** Statistical treatment of the experimental data was carried out by means of one-way analysis of variance (ANOVA) followed by t-test, or, alternatively, by Kruskal-Wallis' ANOVA followed by Mann-Whitney U-test. Probabilities of less than 5% were considered significant.

**Table 1.** Lack of effect of L-5-HTP on drinking motivation and shock sensitivity

<table>
<thead>
<tr>
<th>L-5-HTP (mg/kg IP)</th>
<th>Drinking motivation (ml/10 min)</th>
<th>Shock sensitivity (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.5 ± 0.5 (8)</td>
<td>0.15 ± 0.01 (12)</td>
</tr>
<tr>
<td>50</td>
<td>9.1 ± 0.5 (8)</td>
<td>0.17 ± 0.01 (14)</td>
</tr>
<tr>
<td>100</td>
<td>8.1 ± 0.3 (8)</td>
<td>0.15 ± 0.01 (10)</td>
</tr>
</tbody>
</table>

Water-deprived rats (cf Materials and methods section) were given L-5-HTP (50 or 100 mg/kg IP; 30 min after benserazide, 25 mg/kg IP). Controls (=0) received corresponding vehicle (and benserazide) injections. Thirty minutes later they were placed into individual ‘‘home’’cages and allowed free access to 5.5% glucose solution (drinking motivation experiment), or placed into the conflict test chambers (shock sensitivity experiment). The amount of liquid (ml) consumed during the subsequent 10-min period, or the threshold current (mA) for reacting to electrical shock, respectively, was then determined in the two separate experimental groups. Shown are the means ±SEM of eight (drinking motivation experiment) or 10–14 (shock sensitivity experiment) observations. Statistics: Kruskal-Wallis' ANOVA, H = 2.08, respectively, for the two experiments; no significant differences between treatment groups.