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Interactions of γ-aminobutyric acid and noradrenaline in the high pressure neurological syndrome

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1 The effects on the high pressure neurological syndrome (HPNS) of reducing brain noradrenaline (NA) levels were studied in adult rats. The onset of tremors and convulsions, which occur as pressure is increased, were used as endpoints for assessing the onset and severity of the HPNS.

2 Neonatal treatment with 6-hydroxydopamine (6-OHDA; 100 mg kg^{-1} i.p. alternate days from birth for 2 weeks) which depleted brain NA, produced no change in the HPNS as assessed by the appearance of tremors and convulsions.

3 A second series of NA-depleted rats and equivalent controls were treated with a GABA agonist, muscimol, 0.1 μg intracerebroventricularly. Subsequently the rats were exposed to pressure and the onset and severity of the HPNS was assessed by observation of tremors and convulsions. A combination of NA depletion and intracerebroventricular injection of muscimol significantly raised the onset pressures for tremors and convulsions, i.e. delayed the appearance of the HPNS.

4 These results are consistent with the HPNS being associated with a disturbance in the balance of two or more neurotransmitter systems, rather than simply an increase or reduction in levels of a single transmitter.

Introduction

The high pressure neurological syndrome (HPNS) is the name given to a group of events that occur when man or animals are exposed to increased environmental pressure. It includes such symptoms as EEG (Rostain, 1980; Hugon, Fagni, Rostain & Seki, 1981) and other central neurophysiological changes, tremor, motor incoordination and disorientation (Hunter & Bennett, 1974). In man these events first occur at about 20 atmospheres absolute (ATA) and are currently the limiting factor to deep sea diving. In animals exposed to higher pressures, severe tremor is followed by convulsions and death. The precise pressures at which these occur depends on both compression rate and species (Brauer, Beaver, Hogue, Ford, Goldman & Venters, 1974; Brauer, Beaver, Lahser, Mansfield & Sheehan, 1977) but in mice convulsions appear between 70–100 ATA pressure.

In recent years there has been increasing speculation as to the precise neurochemical basis of HPNS. It is known that some general anaesthetics will delay the onset of the HPNS (Brauer, Goldman, Beaver & Sheehan, 1974) but this is not a universal property of all anaesthetics (Green, Halsey & Wardley-Smith, 1977) and thus prevention of the HPNS cannot simply depend on a central excitation being opposed by a central nervous system depressant. The involvement of catecholamine neurotransmitter systems was first suggested in 1978 when reserpine, which depletes monoamine stores, was shown to lower the compression rate-dependence of the convulsion threshold in mice (Brauer, Beaver, & Sheehan, 1978). The effect of reserpine on the HPNS could be partially reversed by drugs whose actions antagonize specific reserpine effects, such as amphetamine, tranylcypromine and L-tryptophan.

More recently, drugs that selectively deplete different monoamine neurotransmitters were administered to mice, and any resulting changes in the behavioural aspects of HPNS were noted (Koblin, Little, Green, Daniels, Smith & Paton, 1980). No single monoamine was found to be important in the appearance of HPNS and the authors concluded that a balance between different neurotransmitter systems might be more important than absolute neurotransmitter levels.

One neurotransmitter which seems likely to be involved in the appearance of HPNS is γ-

aminobutyric acid (GABA). Certain drugs which are known to facilitate GABA transmission, such as flurazepam, have been shown to raise the thresholds for both tremors and convulsions in mice (Bichard & Little, 1982). These agents were also tested against an intravenous infusion of the known GABA antagonist bicuculline and were found to increase the seizure threshold, the increases being broadly similar to those found with pressure. The authors concluded that treatment with drugs that facilitate GABA transmission will ameliorate the behavioural signs of the HPNS, although these data do not indicate whether a decrease in GABA transmission occurs during the appearance of the HPNS.

This paper describes experiments in which two compounds altering different neurotransmitter systems have been given both separately and together, the neurotoxin 6-hydroxydopamine (6-OHDA) which produces a permanent loss of noradrenergic terminals from those brain regions normally innervated by the dorsal noradrenergic bundle (Clark, Laverty & Phelan, 1972), and the GABA agonist, muscimol.

Methods

Animal preparation

6-Hydroxydopamine Albino rats (Sheffield strain) of either sex were injected intraperitoneally with 100 mg kg^{-1} (as free base) 6-hydroxydopamine hydrochloride (Sigma Ltd.) dissolved in 0.9% w/v NaCl solution (saline) containing ascorbic acid 1 mg ml^{-1} as antioxidant. They were injected on days 1, 3, 5, 7, 9, 11 and 13 after birth. Control rats received similar injections of saline/ascorbate vehicle alone. The animals were then allowed to mature normally. No experiments were carried out before the rats were 8 weeks old.

In view of the problems associated with measuring neurotransmitter levels in brain after rapid decompression, determinations of brain noradrenaline and dopamine levels of similarly treated but non-pressurized litter mates were also carried out by the fluorometric technique of Shellenberger & Gordon (1971). In rats subsequently exposed to pressure, the effectiveness of the 6-OHDA treatment was checked by comparing the sleep time produced by methohexitone for control and treated rats. It has been shown that the duration of anaesthesia produced by methohexitone is markedly potentiated in 6-OHDA-treated rats (Angel & Mason, 1981). Rats were randomly selected from each group and given methohexitone 37.5 mg kg^{-1} intraperitoneally. No animal was pressurized for at least 2 weeks after methohexitone anaesthesia.

Muscimol Muscimol (0.1 μg in 2 μl saline, Sigma Ltd.) was administered intraventricularly to adult albino rats (Sheffield strain) which had previously received either 6-OHDA or saline/ascorbate vehicle. The injections were performed under light halothane/oxygen anaesthesia and control animals received saline alone. All injections were carried out at least 30 min before compression but in all experiments compression was commenced within 50 min of injection. A minimum of 20 min from waking was allowed for recovery from halothane.

Pressurisation and endpoints

All experiments were carried out in a 25 litre pressure chamber, with a maximum pressure rating of 400 ATA. Compression was with helium, at a rate of 3 ATA min^{-1}. Oxygen partial pressure was kept constant at 0.5 ATA, and carbon dioxide was removed with a soda lime absorber. Gases were mixed by means of an induction motor fan, and chamber temperature was adjusted so as to maintain the normal rectal temperature of each rat (38 ± 1°C; Green, 1979).

The severity of the HPNS was assessed using three endpoints: initial tremor, continuous tremor and the first convulsion. Tremor was measured using a small strain gauge which has been described in detail else-

Table 1 Effects of 6-hydroxydopamine (6-OHDA) injections on subsequent regional brain catecholamine concentrations in rats

Region	*Control*	*6-OHDA*	P
Noradrenaline			
Cortex/hippocampus	319 ± 4.2	10.8 ± 1.7	<0.001
Hypothalamus	1513 ± 94.6	1431 ± 44.4	NS
Dopamine			
Hypothalamus	349 ± 78.7	294 ± 79.3	NS
Striatum	7106 ± 625	7068 ± 1215	NS

Results shown as mean ± s.e.mean of catecholamine concentrations expressed as ng g^{-1} wet wt. $n = 3$ in each group.

where (Baker, Halsey, Wardley-Smith & Wloch, 1981). Briefly, it consists of a strain gauge mounted beneath a small cage in which the rat was restrained only by its tail. The signal from the strain gauge was amplified and recorded on magnetic tape; in addition the signal was observed on an oscilloscope. With this technique, the onset pressure for initial tremor could be accurately determined, and was defined as the pressure at which tremor occurred for periods greater than 500 ms. Continuous tremor was defined as tremor which continued for more than 2 min, and the convulsion threshold pressure was taken to be that at which the first tonic-clonic seizures occurred.

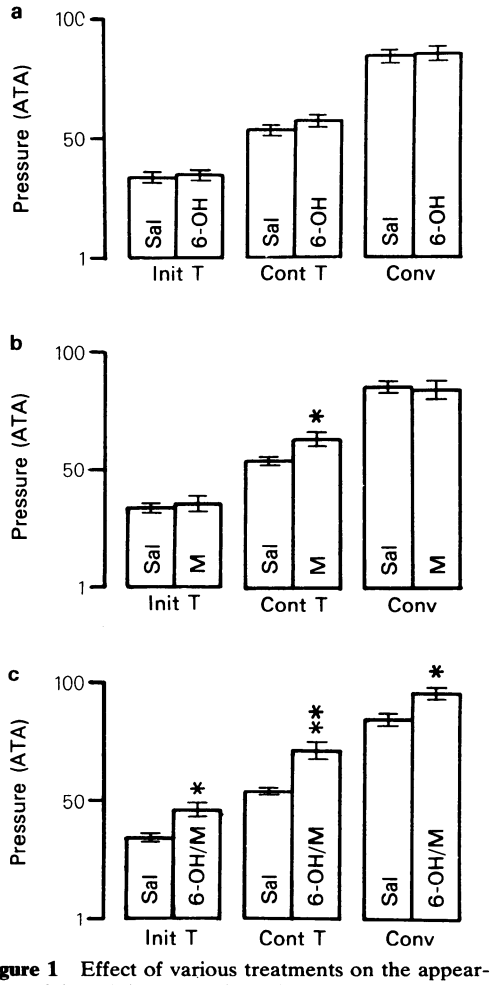

Figure 1 Effect of various treatments on the appearance of HPNS in rats. Init T: initial tremor pressure (ATA) Cont T: continuous tremor pressure (ATA) Conv: convulsion pressure (ATA). All columns show ± 1 s.e.mean (a) Results for saline ($n = 12$) compared with 6-hydroxydopamine (6-OHDA) ($n = 12$). There were no significant differences. (b) Results for saline ($n = 12$) compared with muscimol ($n = 5$). There were no differences for initial tremor or convulsion pressure. Continuous tremor pressure was increased with muscimol. $^*P < 0.01$. (c) Results for saline ($n = 12$) compared with 6-OHDA + muscimol ($n = 6$). All endpoints were significantly increased: $^*P < 0.005$; $^{**}P < 0.001$. Sal = saline; 6-OH = 6-OHDA; M = muscimol; 6-OH/M = 6-OHDA + muscimol.

Experimental protocols

In all experiments, rats were compressed singly, and the onset pressures for the HPNS were recorded. Three series of experiments were carried out with 5–12 rats in each group (each animal was used once only): (1) 6-hydroxydopamine-treated rats v. saline controls. (2) Muscimol-treated rats v. saline controls. (3) 6-Hydroxydopamine + muscimol-treated rats compared with saline controls.

All experiments were carried out between 10 h 00 min and 16 h 00 min. In addition to the measurement of tremor, each rat was continuously observed. At the end of each experiment the rat was killed with an overdose of nitrous oxide prior to decompression. Results were analysed by Student's *t* test (2 tailed) to compare treated with control animals after checking for a normal distribution using the Shapiro-Wilk test.

Results

Preliminary experiments confirmed that there were no differences between male and female rats for any of the treatment regimes. Data for both sexes were subsequently combined. The results from the experiments with methohexitone indicated that in all cases the mean sleep time of the rats treated with 6-OHDA was increased by at least 100%. Control rats had a range of sleep times after methohexitone from 4.25–5.5 min, compared with 6-OHDA-treated rats who had a range of sleep times from 11.0–15.75 min. Estimation of brain noradrenaline and dopamine levels in similarly treated rats are shown in Table 1, and show that noradrenaline levels in cortex and hippocampus were only 3% of control levels whereas that for hypothalamic areas was normal. Dopamine levels were unchanged in the striatum and hypothalamus.

6-Hydroxydopamine

Results for 6-OHDA are shown in Figure 1a. It can be seen that for each of the thresholds used for assessing HPNS there was no significant difference between the rats treated with 6-OHDA and the saline/ascorbate controls.

Muscimol

Results for muscimol administered i.c.v. are shown in Figure 1b. There were no significant differences for either initial tremor or convulsion threshold for muscimol compared with saline controls. However, muscimol raised the threshold for the onset of continuous tremor from 54 ATA in controls to 62.7 ATA in the treated rats. This is an increase of 16% ($P < 0.01$).

6-Hydroxydopamine plus muscimol

Results for pre-treatment with both 6-OHDA and muscimol are shown in Figure 1c. For each of the endpoints there is a significant increase in the treatment groups. Initial tremor threshold was increased by 34% from 34.1 ATA (controls) to 45.6 ATA ($P < 0.005$); continuous tremor threshold also increased by 34% from 54.0 ATA to 72.1 ATA ($P < 0.001$). Convulsion threshold pressure was also raised from 84.9 ATA to 96.6 ATA ($P < 0.005$) although this increase (14%) is less than for tremor threshold pressure.

Discussion

These results support the currently accepted idea that the HPNS is influenced by the interaction of different neurotransmitter systems. Our results show that depletion of noradrenaline in the cortex of the brain has no effect on the appearance of tremors and convulsions due to high pressure. However, when the GABA agonist, muscimol, is administered to the noradrenaline-depleted rats, tremors and convulsions do not occur until a higher pressure has been reached, indicating a protective effect. Muscimol was less effective when used alone when only one threshold, that of continuous tremor, was significantly increased.

Our results with 6-OHDA are in contrast to those of Bowser-Riley, Dobbie, Paton & Smith (1982). In their study, mice were injected intracerebroventricularly with 6-OHDA. The neurotoxin was given acutely immediately before the exposure to pressure. The authors found that treatment with 6-OHDA reduced the convulsion threshold by 25%; their results on tremor threshold were more complex, with fine tremor onset pressure remaining unaffected. However, it is possible that the differences between these and our results can be explained simply on the basis of a different species of animal (mice v. rats) and the different techniques. The relative levels of noradrenaline are unlikely to be identical after acute administration directly into the brain with those obtained by chronic neonatal treatment and this makes interpretation of their results more difficult. Chronic systemic administration of 6-OHDA to neonatal rats produces a widespread and long-lasting depletion of noradrenaline in different regions of the brain, especially cortex, cerebellum and spinal cord (Clark *et al.*, 1972).

Our results showing that muscimol alone has no effect on the HPNS are in agreement with those of Bichard & Little (1982), who found no dramatic effect of muscimol on the HPNS in mice. These authors did not attempt to distinguish the different aspects of tremor seen in mice and found no significant effect on either tremors or convulsions. In our studies we found a significant effect of muscimol only on the continuous tremor threshold pressure. Although we have no explanation for this, it is consistent with the idea that different manifestations of the HPNS have different sites of origin in the brain (Rowland-James, Wilson & Miller, 1981; Bowser-Riley, Paton & Smith, 1981). It is not clear why muscimol, which is considered to be an effective GABA-agonist (Meldrum, 1981) is not more effective in reducing the severity of the HPNS, since drugs which enhance GABA transmission have been shown to be beneficial. However, it is possible that these drugs, such as flurazepam and sodium valproate owe their effectiveness to mechanisms other than those involving GABA. It may be relevant to note that sodium valproate is known to affect excitatory neurotransmitters such as aspartate, which could account for its effect on the HPNS (Chapman, Riley, Evans & Meldrum, 1982).

Although these data indicate that manipulation of two neurotransmitters protects against the HPNS, they do not provide any indication as to the region of the central nervous system involved. Projections of noradrenergic nerves arising from the locus coeruleus are found in the cerebellum, mid brain, thalamus, hypothalamus and cortex (Lindwall & Bjorklund, 1974). GABA is also widely distributed throughout the brain (Fahn & Côté, 1968); thus no conclusions about the probable location in the brain for an interaction of noradrenaline and GABA in the origins of the HPNS can be drawn.

In the absence of this information as to the site of generation of movements at high pressure, it is, at first sight, inexplicable why two putative inhibitory transmitters should give a measure of protection when they are both changed, but in opposite ways. The observations of the present paper lead to the possible conclusion that one of the causes of the HPNS is over-activity in a noradrenergic system which normally functions to inhibit motoneuronal discharge. However, blockade of this system in isolation appears to be inadequate to prevent the signs of the HPNS, and for any marked beneficial effect to be seen it is necessary simultaneously to increase GABA-ergic activity.

In conclusion, these data provide further support for the view that the HPNS is probably associated with changes in more than one neurotransmitter system. We have found that a combination of treatment with 6-OHDA and muscimol significantly raises the threshold pressure at which the behavioural aspects of the HPNS occur. However, this pharmacological intervention may be merely treating the symptoms of the HPNS rather than influencing its genesis, and so the exact neurochemical events taking place both during the HPNS and during its amelioration with drugs remains to be elucidated.

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