PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Specific Effects of Drugs at Pressure: Animal Investigations [and Discussion]

A. Angel, M. J. Halsey, Hilary Little, B. S. Meldrum, J. A. S. Ross, J.-C. Rostain, Bridget Wardley-Smith, H. J. Little, W. D. M. Paton, J.-C. Rostain and Bridget Wardley-Smith

Phil. Trans. R. Soc. Lond. B 1984 **304**, 85-94
doi: 10.1098/rstb.1984.0010

Phil. Trans. R. Soc. Lond. B **304**, 85–94 (1984) [85]
Printed in Great Britain

Specific effects of drugs at pressure: animal investigations

By A. Angel[1], M. J. Halsey[2]†, Hilary Little[3], B. S. Meldrum[4], J. A. S. Ross[2], J.-C. Rostain[5] and Bridget Wardley-Smith[2]

[1]*Department of Physiology, University of Sheffield, Sheffield S10 2TN, U.K.*
[2]*H.p.n.s. Group, Clinical Research Centre, Watford Road, Harrow, Middx HA1 3UJ, U.K.*
[3]*Department of Pharmacology, University of Oxford, South Parks Road, Oxford OX1 3QT, U.K.*
[4]*Department of Neurology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, U.K.*
[5]*G.I.S. Physiologie Hyperbare, C.N.R.S. Faculté de médecine nord, 13326, Marseille, Cedex 15, France*

The interactions of anaesthetics and other drugs with high pressure suggest that protection against the high pressure neurological syndrome (h.p.n.s.) can no longer be considered in terms of generalized non-specific mechanisms. The evidence from our work shows that anaesthetics may either protect, have no effect, or potentiate h.p.n.s. Structural analogues of the steroid anaesthetic Althesin have a protective effect against high pressure tremors in spite of the fact that they have no anaesthetic effects. Low doses of flurazepam are effective against tremor but can be antagonized by Ro 15-1788, which implies in this case a role for the benzodiazepine receptor complex.

Pressure interactions with other drugs have included the classic anticonvulsants – which, in general, were relatively ineffective – and various agents perturbing the balance of specific neurotransmitter systems. Representative examples from different studies include 6-hydroxydopamine, muscimol, and sodium valproate. Finally, the potent protection against h.p.n.s. by 2-amino-phosphonoheptanoic acid, an antagonist with preferential action against excitation produced by aspartate and *N*-methyl-D-aspartate, provides the first evidence that enhanced *excitatory* amino acid neurotransmission may have an important role in the h.p.n.s.

Introduction

Interactions between many drug and pressure induced effects were extensively investigated by F. H. Johnson and H. Eyring in the 1940s and 1950s, by using luminous bacteria as their primary biological system (Johnson *et al.* 1954). Their data were interpreted in terms of enzyme kinetics. Subsequent work with both tadpoles (Johnson & Flagler 1950) and newts (Lever *et al.* 1971) concentrated on anaesthetic–pressure interactions, both in terms of the pressure reversal of anaesthesia and the anaesthetic amelioration of the high pressure neurological syndrome (h.p.n.s.). However, in 1975 it was recognized that a wider pharmacological approach to the study of pressure might prove rewarding (Halsey *et al.* 1975) and this paper reviews some of the contributions to the subject since that date.

Pressure reversal of anaesthesia

The majority of the work on the pressure reversal of anaesthesia has been in animals, mainly rodents. This has raised the obvious problem that the relevance of the animal models to the

Correspondence should be addressed to Dr M. J. Halsey at the Clinical Research Centre, Harrow.

human diver is unproven. However, a recent study on the pressure reversal of Althesin anaesthesia in man (Dundas 1979) has provided the opportunity for a quantitative comparison with the same phenomenon in rats (Halsey *et al.* 1978). There is a surprisingly good correlation between the two effects (Dundas *et al.* 1982), which suggests that the rat is not an unacceptable model system for pharmacological studies at pressure.

Although the phenomenon of the pressure-reversal of anaesthesia has been known for many years, it has been difficult to establish whether this behavioural observation is simply the sum of many different perturbations of the central nervous system or a direct interaction between the anaesthetic and pressure effects. A study on the pressure reversal of the effect of urethane on the evoked somatosensory cortical response in the rat demonstrated a direct link between electrophysiological and behavioural studies (Angel *et al.* 1980). The cerebral response evoked by stimulation of the forepaw in the rat showed an increase in latency and a decrease in amplitude of its initial components as the anaesthetic dose of urethane was increased. These changes were reversed if the ambient pressure was increased with helium up to 100 atm (absolute)†. The dose of urethane required to prevent response to tail stimulation was also increased as the pressure increased. The magnitudes of the pressure reversal of the two measurements were surprisingly similar (a variation of less than 14% over the 100 atm (absolute) range). So high ambient pressures not only restore the motor responsiveness of the central nervous system, they also appear to restore its sensory responsiveness.

Anaesthetic interactions with the h.p.n.s.

Studies on anaesthetic amelioration of h.p.n.s. have now been extended from the gaseous agents to a wide range of intravenous agents with differing chemical structure. All gaseous agents studied in mice or rats have proved effective and the only agent so far studied in man (nitrogen) is consistent with the prediction of the animal investigations (reviewed in Halsey (1982)). The preliminary screening of potentially suitable intravenous drugs, with tadpoles as the animal model, suggested that these too would ameliorate the syndrome (Halsey & Wardley-Smith 1975). However, the quantitative studies in rats, in which drugs are administered by a variable continuous infusion at pressure, have provided unexpected results. Some agents protected against h.p.n.s., others had no observable effect, while others exacerbated the syndrome.

Althesin (Bailey *et al.* 1977) and subsequently methohexitone, thiopentone, propanidid, and ketamine (Green *et al.* 1977) were studied initially. All these anaesthetics required an increase in dose to maintain anaesthesia at pressure, but the amount of extra anaesthetic required varied considerably between agents (Halsey *et al.* 1978). There were also differences in their pressure-protection characteristics: methohexitone and propanidid reduced the convulsion threshold and thiopentone had no statistically significant effect; whereas Althesin and ketamine were very good at preventing convulsions up to 100 atm (absolute) and 170 atm (absolute), respectively. Althesin and ketamine were effective at 50% of their anaesthetic dose; ketamine was particularly good, as it also eliminated tremors. The effects of these drugs on tremor exhibit a slightly different pattern (figure 1). The threshold pressures for the onset of tremors were raised from 37 to 73 atm (absolute) with Althesin, and from 37 to 100 atm (absolute), with ketamine (100 atm was the maximum possible pressure of the

† 1 atm = 101325 Pa.

chamber available at that time). Because both tremors and convulsions are known to disappear with time, the return of h.p.n.s. on withdrawal of the drug indicated that the observed beneficial effects of ketamine and Althesin were real and that the animals were not merely acclimatizing to high pressure. Pressure protection, therefore, is not a universal property of all anaesthetics, and there is no correlation between pressure reversal and pressure protection.

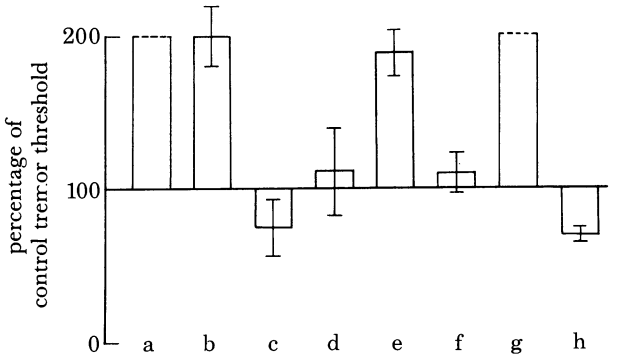

FIGURE 1. The threshold pressures for the onset of tremors expressed as a percentage of the control tremor threshold pressure in the presence of the following drugs: a, ketamine; b, Althesin; c, methohexitone; d, thiopentone; e, minaxalone; f, propanidid; g, nitrous oxide (N_2O); h, morphine. The error bars represent ± 1 s.e.

More recently we have studied minaxalone (11α-*N*,*N*-dimethylamino-2β-ethoxy-3α-hydroxy-5α-pregnan-20-one), which is a new steroid anaesthetic exhibiting properties similar to those of alphaxalone, the major active component of Althesin. Minaxalone is water soluble, so that its formulation does not require Cremophor EL, the vehicle used in Althesin. The anaesthetizing concentration of minaxalone raised the control tremor threshold (figure 1) but the agent was not as effective as Althesin. So, there appear to be significant differences between the steroids in their pressure protection characteristics. This is not unexpected, since one of the first experiments after the discovery of Althesin's anti-h.p.n.s. action was the demonstration that this was not a general property of all steroids. For example, hydrocortisone gave no pressure protection in the tadpole model system (Halsey & Wardley-Smith 1975). Finally, data on morphine at analgesic doses (3.5 mg/kg; intravenous) have been included in figure 1. This drug was investigated as part of a study on the possible role of opioid receptors in h.p.n.s. It is known, for example, that naloxone will prevent drug-related convulsions in mice and antagonize ketamine and nitrous oxide analgesia (two agents that are particularly effective in ameliorating h.p.n.s.). The preliminary results of this investigation (Wardley-Smith *et al.* 1981) suggest that opioid receptors play only a minor role, if any, in h.p.n.s., and it is clear that morphine exacerbates rather than ameliorates the syndrome.

NON-ANAESTHETIC DRUGS INTERACTING WITH H.P.N.S.

The most effective anti-h.p.n.s. compounds so far are certain general anaesthetics, but there are some interesting examples outside this group of compounds that may have important mechanistic implications. The initial investigations with non-anaesthetic anti-h.p.n.s. compounds were disappointing. A study in which anticonvulsant drugs were screened for anti-h.p.n.s. activity in mice showed that only those compounds having some sedative action at high concentrations (for example, diazepam) appeared to have any significant value (Halsey &

Wardley-Smith 1981). Thus classical anticonvulsants, such as ethosuximide or carbamazepine, did not raise the threshold pressure for the onset of the signs of h.p.n.s. However, one of the benzodiazepines that was subsequently found to be effective, flurazepam (Bichard *et al.* 1981), has now been investigated in more detail. The benzodiazepine antagonist Ro 15-1788 has been shown to block selectively the actions of benzodiazepines, which are thought to be mediated via specific benzodiazepine receptors, but it does not antagonize the non-specific actions of large doses of benzodiazepines. Bichard & Little (1982*a*) have found that administration of this antagonist completely prevented the protective effect of flurazepam as assessed by threshold pressures for the onset of tremors and convulsions in mice. An important aspect of this work was that Ro 15-1788 injected alone at the effective concentration did not alter the onset pressures. These results suggest that the effects of flurazepam on the behavioural responses to high pressure are mediated through its action on benzodiazepine receptors, rather than via non-specific action on cell membranes.

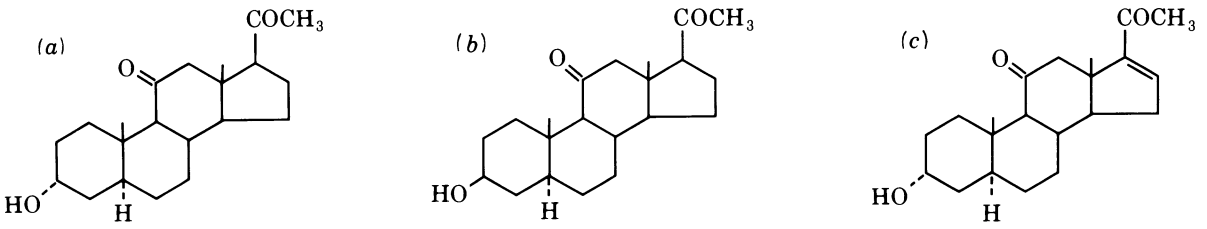

FIGURE 2. The structures of (*a*) 3α-hydroxy-alphaxalone, the major active constituent of the anaesthetic Althesin, and its structural analogues, (*b*) 3β-hydroxy-alphaxalone and (*c*) Δ-16-alphaxalone, both of which have no anaesthetic properties.

A final example of non-anaesthetic drugs interacting with h.p.n.s. is provided by the steroids structurally related to the anaesthetic alphaxalone. It was known that alphaxalone (the principal active component of the clinical anaesthetic Althesin) was particularly effective against h.p.n.s. However, structural analogues of alphaxalone-3-β-hydroxy-alphaxalone and Δ-16-alphaxalone (figure 2) are completely non-anaesthetic and have no recognized pharmacological actions. Either of these compounds, infused during continuous pressurization of rats, ameliorated the severe tremors associated with h.p.n.s. without any shift in the tremor frequency (11–14 Hz). Tremor returned, usually about 3 min after the initial drug infusion was stopped (Halsey & Wardley-Smith 1983).

These and other data suggest that amelioration of h.p.n.s. is not necessarily associated with anaesthesia, but rather is a property of individual anaesthetic and non-anaesthetic compounds. It is always difficult to exclude a component of non-specific action (for example, general membrane contraction and expansion) in the effects of any drug, but the data reviewed here emphasize the importance of also considering the more selective actions of drugs at pressure.

CATECHOLAMINE AND GABA NEUROTRANSMITTER SYSTEMS

One of the obvious areas of selective actions of drugs is their interaction with various neurotransmitter systems. There has been increasing speculation on the relative roles of the different systems in h.p.n.s. 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 *et al.* 1978). The effect of reserpine on the h.p.n.s. could be partially reversed by drugs, such as amphetamine tranylcypromine and L-tryptophan, whose actions antagonize specific reserpine effects.

More recently, drugs that deplete different monoamine neurotransmitters were administered to mice and any resulting changes in the behavioural aspects of h.p.n.s. were noted (Koblin *et al.* 1980). No single monoamine was found to be important in the appearance of h.p.n.s. and the authors concluded that a balance between different neurotransmitter systems might be more important than absolute neurotransmitter levels.

TABLE 1. NORADRENALIN OR GABA INTERACTIONS, OR BOTH, WITH H.P.N.S.

	initial tremor (pressure ± s.e.)	continuous tremor (pressure ± s.e.)	convulsions (pressure ± s.e.)
treatment	atm (absolute)	atm (absolute)	atm (absolute)
saline	32.4 ± 2.4	56.4 ± 1.8	85.3 ± 1.5
6-OHDA	33.1 ± 1.4	61.0 ± 2.3	87.8 ± 3.0
muscimol	34.5 ± 3.4	63.4 ± 2.7	81.4 ± 4.6
muscimol with 6-OHDA	44.0 ± 1.8†	75.0 ± 2.5‡	97.0 ± 3.2†

The effects of chronic pretreatment with 6-hydroxydopamine (6-OHDA) or acute intraventricular pretreatment with muscimol, or both, on three behavioural end points of the h.p.n.s. († $p < 0.01$, ‡ $p < 0.001$).

One neurotransmitter, which seems likely to be involved in the appearance of h.p.n.s., is γ-aminobutyric acid (GABA). Certain drugs that are known to facilitate GABA transmission have been shown to raise the thresholds for both tremors and convulsions in mice (Bichard & Little 1982*b*). 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 h.p.n.s., although these data do not indicate whether a decrease in GABA transmission occurs during the appearance of the h.p.n.s.

The interaction between GABA and noradrenalin in the h.p.n.s. has now been studied in rats (Angel *et al.* 1983). Two compounds altering different neurotransmitter systems have been given both separately and together; the neurotoxin 6-hydroxydopamine, which produces a permanent loss of noradrenergic terminals from those brain regions normally innervated by the dorsal noradrenergic bundle, and the GABA agonist, muscimol. The 6-hydroxydopamine was injected on alternate days from birth for two weeks. Subsequent regional brain catecholamine measurements demonstrated that this pretreatment selectively and permanently reduced the noradrenalin levels in the cortex–hippocampus. The results (table 1) indicated that this depletion had no effect on the appearance of tremor or convulsions due to high pressure. These data are in contrast with other preliminary experiments using 6-hydroxydopamine administered one to six days before compression (Bowser-Riley *et al.* 1982). However, the reasons for the difference between the two results are speculative.

The GABA agonist, muscimol, administered intraventricularly to the adult untreated rat had no major effect on h.p.n.s. (table 1). This is in accord with earlier work in mice (Bichard & Little 1982*b*). However, the administration of muscimol to the noradrenalin-depleted animals produced a marked protective effect. These data indicate that manipulation of two

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

neurotransmitter systems protects against the h.p.n.s. It would be unjustified to draw any conclusions as to the regions of the central nervous system involved, especially since such pharmacological intervention may be merely treating the symptoms of the h.p.n.s. rather than influencing its genesis.

A final example of neurotransmitter manipulation is provided by one drug, sodium valproate, which is currently being studied in both rats and baboons. This drug is a clinical antiepileptic agent that is known to produce a number of biochemical changes including increases in GABA levels, primarily by inhibition of the GABA–transaminase reaction. It should be noted that it is not only this neurotransmitter that is affected and, for example, one additional hypothesis is that excitatory transmission, especially that mediated by aspartate, is reduced by valproate (Meldrum 1980). The mechanisms of the anticonvulsant action of valproate still await clarification by a variety of biochemical and neurophysiological techniques (Chapman *et al.* 1982). However, the interest in this compound in the hyperbaric field was initiated by the demonstration that prior administration raised the threshold pressures for both tremors and convulsions in mice (Bichard *et al.* 1981). Subsequent experiments have now been done in rats, in which it has been possible to make both behavioural and e.e.g. observations (Rostain *et al.* 1983). Injection of valproate before compression had a greater protective effect on tremor (onset pressure increased from 40 to 72 atm (absolute)) compared with myoclonia (onset pressure increased from 77 to 90 atm (absolute)). Convulsions were not seen up to the maximum pressure (100 atm (absolute)) of these experiments with sodium valproate. The drug modified the power spectra of the awake e.e.g. activities at normal pressure and these modifications persisted at pressure. More importantly, the increase in 4–7 Hz waves normally observed during compression was not observed with sodium valproate at pressure.

The previous work exposing baboons to high pressure has demonstrated one of the problems of interpreting behavioural observations. The e.e.g. associated with a high pressure convulsion with a helium and oxygen breathing mixture indicates a generalized seizure, whereas the convulsion occurring at a higher pressure after the addition of nitrogen to the breathing mixture indicates a more localized seizure in the occipital region (Rostain 1980). So pharmacological intervention – in this case with nitrogen – may change the nature of the hyperbaric response as well as altering its onset pressure.

Preliminary experiments are now being done to study the effects of sodium valproate in the baboon. The use of this animal model not only allows more detailed physiological measurements to be made but is also thought to represent the best model for man. Sodium valproate has been administered orally from a period of 3 days to 2 weeks before the dive. Serum measurements have demonstrated that, with the longer periods of treatment, significant levels of the drug have been attained. The 2 week chronic treatment, which was continued during the dive, resulted in a significant amelioration of tremor in the baboons up to the maximum pressure studied so far (40 atm (absolute)). So sodium valproate has now been demonstrated to be effective in mice, rats and baboons. More detailed studies are now being made, but the exact neurochemical events during the h.p.n.s. and during its amelioration with drugs remains to be elucidated.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Excitatory amino acid antagonists

A wide range of compounds has recently been evaluated as antagonists of excitatory amino acids (Watkins & Evans 1981). One of these, 2-amino-7-phosphonoheptanoic acid (2 APH), has been shown to have a dramatic protective effect against h.p.n.s. (Meldrum *et al.* 1983). The effect on tremor, myoclonus and convulsions is illustrated in figure 3. 2-APH is a selective antagonist of excitation produced by *N*-methyl-D-aspartic acid (NMDA), but with minimal

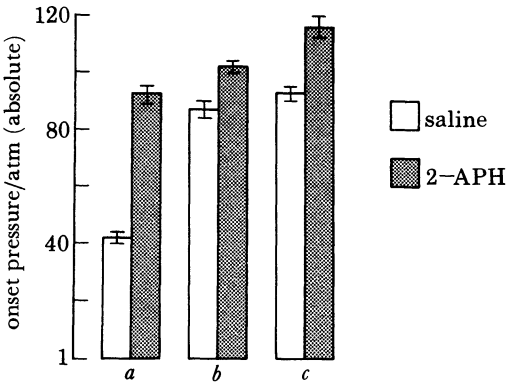

Figure 3. The onset pressure for (*a*) initial tremor; (*b*) myoclonus; (*c*) convulsion, after pretreatment with either saline or 2-amino-7-phosphonoheptanoic acid, 2-APH. The error bars represent ±1 s.e.

activity against excitation produced by kainic acid or quisqualic acid (Evans *et al.* 1982; Perkins *et al.* 1981). It has already been demonstrated to have anticonvulsant activity when tested against audiogenic and chemically induced seizures (Croucher *et al.* 1982; Czuczwar & Meldrum 1982). In the pressure experiments (Meldrum *et al.* 1983) the most dramatic effect was on tremor (it increased the threshold pressure by a factor of 2.2). The tremor that did occur at the higher pressures was much less severe in the 2-APH group compared with their saline controls, remaining both mild and intermittent up to the convulsion onset pressure. The percentage changes in tremor onset pressures seen with 2-APH were greater than has previously been described with the most effective non-anaesthetic agents, such as sodium valproate and flurazepam (Bichard & Little 1982*b*). Although highly significant, the effect of 2-APH on both myoclonus and convulsions was less pronounced than the effect on tremor. These data support the view that the different phases of the h.p.n.s. have different sites of origin.

Further experiments are in progress with a less specific excitatory amino acid antagonist, *cis*-2,3-piperidine dicarboxylic acid (PDA), which also antagonizes excitation produced by NMDA, but to a lesser extent than 2-APH. In addition it antagonizes excitation due to kainic and quisqualic acid. The protective effects of PDA against myoclonus and convulsions exactly mirrored those produced by 2-APH. However, it was much less effective against tremor, although there was still significant improvement.

These potent protective actions of agents acting post-synaptically to decrease excitation produced by aspartate and related compounds provides the first evidence that enhanced excitatory neurotransmission may contribute importantly to the h.p.n.s. Aspartate is probably the transmitter released by climbing fibres in the cerebellum, and by certain interneurons in

the spinal cord (Watkins & Evans 1981). Abnormal function in aspartergic synapses could be a causal factor in the tremor of the h.p.n.s. The use of excitatory amino acid antagonists offers a new pharmacological approach to the prophylaxis of the syndrome in man.

References

Angel, A., Gratton, D. A., Halsey, M. J. & Wardley-Smith, B. 1980 Pressure reversal of the effect of urethane on the evoked somatosensory cortical response in the rat. *Br. J. Pharmac.* **70**, 147–241.

Angel, A., Halsey, M. J. & Wardley-Smith, B. 1983 Interactions of γ-amino butyric acid and noradrenaline in the high pressure neurological syndrome. *Br. J. Pharmac.* **79**, 725–729.

Bailey, C. P., Green, C. J., Halsey, M. J. & Wardley-Smith, B. 1977 High pressure and intravenous steroid anaesthesia in rats. *J. appl. Physiol respir. exercise Physiol.* **43**, 183–188.

Bichard, A. R. & Little, H. J. 1982*a* The benzodiazepine antagonist, Ro-15-1788, prevents the effects of flurazepam on the high pressure neurological syndrome. *Neuropharmacology* **21**, 877–880.

Bichard, A. R. & Little, H. J. 1982*b* Drugs that increase GABA transmission protect against the HPNS. *Br. J. Pharmac.* **76**, 447–452.

Bichard, A. R., Little, H. J. & Paton, W. D. M. 1981 The involvement of GABA in the high pressure neurological syndrome (HPNS). *Br. J. Pharmac.* **74**, 221*P*.

Bowser-Riley, F., Dobbie, J. A., Paton, W. D. M. & Smith, E. B. 1982 A possible role for monoaminergic inhibition in the high pressure nervous syndrome. *Undersea biomed. Res.* **9** (suppl.), 32–33.

Brauer, R. W., Beaver, R. W. & Sheehan, M. E. 1978 The role of monoamine neurotransmitters in the compression rate dependence of HPNS convulsions. In *Underwater Physiology 6*. Proceedings of the sixth symposium on underwater physiology (ed. C. W. Shilling & M. W. Beckett), pp. 49–60. Bethesda: Federation of American Societies for Experimental Biology.

Chapman, A., Keane, P. E., Meldrum, B. S., Simiand, J. & Vernieres, J. C. 1982 Mechanism of anticonvulsant action of valproate. *Prog. Neurobiol.* **19**, 315–359.

Croucher, M. J., Collins, J. F. & Meldrum, B. S. 1982 Anticonvulsant action of excitatory amino acid antagonists. *Science, N.Y.* **216**, 899–901.

Czuczwar, S. J. & Meldrum, B. S. 1982 Protection against chemically induced seizures by 2-amino-7-phosphonoheptanoic acid. *Eur. J. Pharmac.* **83**, 335–338.

Dundas, C. R. 1979 Alphaxalone/alphadalone in diving chamber anaesthesia. *Lancet* vol. i, 378.

Dundas, C. R., Halsey, M. J. & Wardley-Smith, B. 1982 Anaesthesia at pressure – the rat as a model for man. *Undersea biomed. Res.* **9** (suppl.), 41.

Evans, R. H., Francis, A. A., Jones, A. W., Smith, D. A. S. & Watkins, J. C. 1982 The effects of a series of ω-phosphonic δ-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. *Br. J. Pharmac.* **75**, 65–75.

Green, C. J., Halsey, M. J. & Wardley-Smith, B. 1977 Possible protection against some of the physiological effects of high pressure. *J. Physiol., Lond.* **267**, 46P–47P.

Halsey, M. J. 1982 Effects of high pressure on the central nervous system. *Physiol. Rev.* **62**, 1342–1377.

Halsey, M. J., Settle, W. & Smith, E. B. (eds) 1975 *The strategy for future diving to depths greater than 1000 feet*. Bethesda: Undersea Medical Society.

Halsey, M. J. & Wardley-Smith, B. 1975 Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquillizers. *Nature, Lond.* **257**, 811–813.

Halsey, M. J. & Wardley-Smith, B. 1981 The high pressure neurological syndrome: do anticonvulsants prevent it? *Br. J. Pharmac.* **72**, 502*P*–503*P*.

Halsey, M. J. & Wardley-Smith, B. 1983 Non-anaesthetic steroids ameliorate the high pressure neurological syndrome in rats. *Neuropharmacology* **22**, 103–108.

Halsey, M. J., Wardley-Smith, B. & Green, C. J. 1978 The pressure reversal of general anaesthesia – a multi-site expansion hypothesis. *Br. J. Anaesth.* **50**, 1091–1097.

Johnson, F. H., Eyring, H. & Polissar, M. J. 1954 *The kinetic basis of molecular biology*. New York: Wiley.

Johnson, F. H. & Flagler, E. A. 1950 Hydrostatic pressure reversal of narcosis in tadpoles. *Science, N.Y.* **112**, 91–92.

Koblin, D. D., Little, H. J.; Green, A. R., Daniels, S., Smith, E. B. & Paton, W. D. M. 1980 Brain monoamines and HPNS. *Neuropharmacology* **19**, 1031–1038.

Lever, M. J., Miller, K. W., Paton, W. D. M. & Smith, E. B. 1971 Pressure reversal of anaesthesia. *Nature, Lond.* **231**, 368–371.

Meldrum, B. S. 1980 Mechanism of action of sodium valproate. *Brain Res. Bull.* **5**, 579–584.

Meldrum, B., Wardley-Smith, B., Halsey, M. & Rostain, J.-C. 1983 2-amino-phosphonoheptanoic acid protects against the high pressure neurological syndrome. *Eur. J. Pharmac.* **87**, 501–502.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Perkins, M. N., Stone, T. W., Collins, J. F. & Curry, K. 1981 Phosphonate analogues of carboxylic acids as aminoacid antagonists on rat cortical neurones. *Neuroscience Lett.* **23**, 333–336.
Rostain, J.-C. 1980 Le syndrome nerveux des hautes pressions chez l'homme et le singe *Papio papio*. D.Sc. thesis, Université d'Aix-Marseille I., Marseille, France.
Rostain, J.-C., Wardley-Smith, B. & Halsey, M. J. 1983 Effects of sodium valproate on HPNS in rats: the probable role of GABA. In Proceedings 8th Symposium on Underwater Physiology. (In the press.)
Wardley-Smith, B., Halsey, M. J. & Ross, J. A. S. 1981 Naloxone, morphine and nitrous oxide interactions at pressure: the role of opioid receptors in HPNS. *Undersea biomed. Res.* **8** (suppl.), 66.
Watkins, J. C. & Evans, R. H. 1981 Excitatory amino acid transmitters. *A. Rev. Pharmac. Toxicol.* **21**, 165–204.

Discussion

H. J. Little (*University Department of Pharmacology, Oxford, U.K.*). I have been asked to present some additional data and to suggest some points for discussion.

We have shown that drugs that potentiate GABA transmission, such as sodium valproate and flurazepam, protect against the h.p.n.s. in small mammals (Bichard & Little 1982*b*) and Dr Halsey has described our results with the specific benzodiazepine antagonist Ro 15-1788. We have also heard that muscle relaxants and glutamate antagonists can also decrease the high pressure syndrome. There are two questions posed by these results. First, what is the mechanism by which the drugs exert their effects? Second, does this provide any information about the underlying mechanism of the syndrome?

We have examined the effects of pressure on GABA transmission *in vitro* and have found no evidence that it is depressed at pressure. The action of GABA at its receptor sites was found to be unaffected by 130 atm of helium (studies with the superior cervical ganglion (Little 1982)) and we have found that GABA release from the frog spinal cord was increased rather than decreased by helium pressure. Admittedly, changes in GABA transmission may be occurring at sites that we have not examined, but I would like to provoke discussion by suggesting that the protection against the h.p.n.s. provided by increasing activity in a particular neurotransmitter system does not necessarily provide any information as to the cause of the h.p.n.s. Similarly, if decreasing activity in a neurotransmitter system decreases the pressure thresholds this might mean that pressure is acting on that system, showing an additive effect. Alternatively, it may mean that pressure is having no effect on that system so that the drug action can cause considerable changes that might not be seen in a system that has been compromised by the effect of pressure.

I thought that there were two other points raised that might be of interest to discuss. First, the importance of the compression rate in pharmacological studies. Dr Brauer showed several years ago that reserpine treatment removes the compression rate dependence of the h.p.n.s. It is interesting to draw an analogy, albeit a speculative one, between this and the involvement of monoamine transmission in adaptive behaviour in animals, such as learning and the acquisition of drug tolerance.

Secondly, there has been considerable discussion today about pressure–anaesthetic interactions, but this reciprocal antagonism has not been seen at any synaptic site. Both Dr Miller's and our own work have shown that the potentiation of GABA transmission, characteristic of some general anaesthetics, such as the barbiturates, is unaffected by high pressure. It may be important in this context to consider various actions of general anaesthetics such as this, which may contribute to the end point measured, usually loss of righting reflex, but which are not pressure reversed, and which may be exerted by an anaesthetic in addition to its 'non-specific'

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

effect. The potentiation of GABA transmission alone does not appear to be sufficient to cause general anaesthesia as the benzodiazepines do not cause loss of responses except at lethal or near-lethal doses (Bichard and Little, Anaesthetics Research Society Meeting, March 1983). Specific effects such as this, however, may explain why results of *in vivo* studies on anaesthesia do not fit simple physico-chemical predictions, but these actions may occur *in addition* to the 'non-specific' mechanism common to all anaesthetics.

Reference

Little, H. J. 1982 *Br. J. Pharmac.* **77**, 209–216.

W. D. M. Paton, F.R.S. In your interesting tests with the heptanoic acid derivative, the animal appeared to show pilo-erection round its snout and salivation. Does the drug have some other actions, for instance a muscarinic effect, at the doses used to control the h.p.n.s.?

B. S. Meldrum. We have also observed hypersalivation in baboons after administration of high doses of the related compound 2-amino-5-phosphonovaleric acid (Meldrum *et al.* 1983).

If assessed by iontophoresis on single units in the spinal cord or cortex, 2-amino-7-phosphonoheptanoic acid and 2-amino-5-phosphonovaleric acid do not possess either muscarinic or anti-muscarinic properties. The salivation may be secondary to a central action of the amino acid antagonists, but we cannot definitively exclude a peripheral muscarinic action.

Reference

Meldrum, B. S., Croucher, M. J., Badman, G. & Collins, J. F. 1983 *Neurosci. Lett.* **39**, 101–104.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES