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THE EFFECTS OF MK801 ON THE HIGH PRESSURE NEUROLOGICAL SYNDROME IN THE BABOON (*PAPIO ANUBIS*)

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Summary—The *in vivo* neurophysiological interactions of the non-competitive NMDA receptor antagonist MK801 with the High Pressure Neurological Syndrome have been investigated in the primate *Papio anubis*. A hyperbaric chamber was used to achieve environmental pressures of 61 ATA (atmospheres absolute) over a period of 5 hr. Eight animals underwent 2 compressions each, one following pretreatment with 0.03 mg/kg (i.v.) MK801, the other a control. Half of the animals received MK801 on their first exposure. Mild signs of the high pressure neurological syndrome, e.g. paw and limb tremor were first observed between 10 and 20 ATA and more severe signs, e.g. whole body tremor, myoclonus and vomiting, appeared after 50 ATA. The onset pressures for the various signs were increased by 10–17 ATA when the animals received MK801 ($P = 0.06$) and the severity of the signs, over the whole range of pressures at which they appeared, was significantly reduced ($P < 0.001$). Additional experiments showed that MK801 afforded considerable protection, at pressures up to 81 ATA, but doses larger than those used for the main experiment produced signs of tranquilisation and sedation.

Changes in the EEG were observed in channels associated with the frontal, parietal and occipital regions. Amplitude and frequency spectra were calculated and trends with pressure in the 4 conventional wavebands were analysed. The most striking change was a decrease in amplitude of delta waves ($P < 0.001$), which was ameliorated by MK801 ($P < 0.001$).

Key words—HPNS, primates, MK801, NMDA receptors, EEG, behavioural observations.

Exposure of man and animals to hyperbaric environments of helium and oxygen leads to disturbances of the nervous system, known as the High Pressure Neurological Syndrome. Signs and symptoms include tremor, myoclonus, motor inco-ordination, sleep disturbances, EEG changes and, in animals exposed to very high pressures, convulsions (Hunter and Bennett, 1974; Brauer, 1975; Halsey, 1982). Pharmacological amelioration of the high pressure neurological syndrome has been effected in both rats (Rostain, Wardley-Smith, Forni and Halsey, 1986) and baboons (Doré, Halsey, Luff, Maclean and Pearce, 1988; Pearce, Clarke, Doré, Halsey, Luff and Maclean, 1989a) by sodium valproate, which has properties affecting both excitatory and inhibitory amino acids in the CNS. In addition it has been shown in rats that competitive antagonism at the *N*-methyl-D-aspartate (NMDA) receptor site afforded greater protection against the high pressure neurological syndrome delaying further the pressure of onset of hyperbaric-induced tremors (Meldrum, Wardley-Smith, Halsey and Rostain, 1983; Wardley-Smith and Meldrum, 1984; Wardley-Smith, Meldrum and Halsey, 1987).

The effects of non-competitive antagonism at the NMDA receptor are, however, less straight forward. Ketamine, at anaesthetic doses in rats, prevented tremors and convulsions, up to 100 ATA (Green, Halsey and Wardley-Smith, 1977), while at sub-anaesthetic doses no effect on tremor was observed, although convulsions were delayed (Wardley-Smith and Wann, 1989). In the baboon, at sub-anaesthetic doses, ketamine accelerated the onset of tremor (J. A. S. Ross, personal communication).

The drug MK801 is a novel anticonvulsant (Clineschmidt, Martin and Bunting, 1982), which acts as a potent and selective non-competitive NMDA antagonist (Wong, Kemp, Priestley, Knight, Woodruff and Iversen, 1986). The baboon model is one of the closest to man and in *Papio papio*, MK801 was very effective in suppressing photo-induced seizures (B. S. Meldrum, unpublished observations). In the experiment reported here, a related species, *Papio anubis*, was used to study the effects of MK801 against pressure-induced hyperexcitability. The model itself (Pearce, Halsey, Ross, Luff, Bevilacqua and Maclean, 1989b) has been developed to allow the animal complete freedom of movement within a cage, yet facilitate direct observations, physiological monitoring and intravenous administration of drugs, under both normal and hyperbaric conditions. It has already been used to investigate the effects of sodium valproate on the high pressure neurological syndrome and work with MK801 is now presented, which is an

extension of studies of the role of excitatory transmitters in the high pressure neurological syndrome.

METHOD

Baboons were exposed to increasing ambient pressures, at which the high pressure neurological syndrome became well established, behavioural and physiological changes were observed and the effects of MK801 on these changes studied.

Pressure chamber

The chamber and ancillary equipment were the same as those used in a previous study (Pearce *et al.*, 1989a). Essentially the chamber was a 1200 l carbon steel cylinder with hemispherical ends, capable of working to 200 ATA. Five portholes allowed 3 direct observation points, an external light source and a television camera. A gas circulating system ensured that the internal atmospheric conditions were kept optimal by removing excess carbon dioxide, water vapour and other contaminants. The compressant gas was helium, which has a high thermal conductivity and so a heating system ensured that the temperature could be controlled to very fine limits.

Food in the form of pellets was available *ad libitum*, as was a supply of drinking water. In addition, a funnel below the cage could be flushed to remove excreta from the chamber, through a pressure lock.

Animals

Eight female adolescent baboons (*Papio anubis*), weighing 5–7 kg, were implanted with subcutaneous monitoring electrodes and arterial and venous catheters, using techniques previously described (Pearce *et al.*, 1989a). The animals wore quilted cloth jackets under a moulded orthoplast waistcoat, to which a backpack was attached. The catheters and wires from the electrodes exited between the shoulder blades and made connections in the backpack. An umbilical of flexible stainless steel tubing then connected the backpack to an electrical and fluid swivel, mounted at the back of the cage. Each catheter remained patent by continuous perfusion with 0.9% sodium chloride at 2 ml hr^{-1}, being supplied from a 500 ml bag of saline in a Tycos pressure infuser, with each line passing through an intraflow valve.

The animal thus had freedom of movement within the cage, whilst monitoring, behavioural observations, arterial blood sampling and intravenous administration of drug took place. Parameters measured were electroencephalogram (EEG) (frontal, parietal and occipital) electro-oculogram (EOG), electrocardiogram (ECG), electromyogram (EMG, trapezium and triceps), subcutaneous temperature, respiration and arterial blood pressure. These were recorded onto both the chart paper of a Siemens Elema mingograph 10 and onto magnetic tape.

Drugs

(+)-5-Methyl-10,11-dihydro-5h-dibenzo(a,d) cyclohepten-5,10-imine (MK801, (4 mg) (Merck, Sharpe & Dohme) was dissolved in 10 ml 0.9% sodium chloride and the resulting solution passed through a 0.2 μm filter into a sterile vessel. Each dose was administered intravenously from outside the chamber and supplied via an HPLC pump (Kontron Instruments). Arterial blood samples were taken both at pressure and during decompression for haematological analysis and measurement of levels of MK801 by radio-immunoassay (Merck, Sharpe & Dohme).

Experimental protocol

The protocol for each animal lasted for 12 weeks and followed that described in an earlier experiment (Pearce *et al.*, 1989a). In brief, the animals went through a training period in order for them to become accustomed to the jackets and tethering system. After this, the operation took place during which the intravascular lines and monitoring electrodes were implanted. Three weeks were allowed for full recovery before the first exposure to pressure.

Compression protocol

Individual baboons vary considerably in their responses to pressure and so each animal was used as its own control. This necessitated 2 exposures for each animal, with recovery in good condition from each.

The protocol for each exposure started with the animal spending 3 days in the chamber with the lid off, in order for it to become accustomed to the general environment. On the fourth day, the intravascular fluid supply was changed from the Tycos infuser to a high pressure liquid chromatography (HPLC) pump, operated from outside the chamber and the lid was bolted down. The environmental partial pressure of oxygen was raised to 0.4 bar and control observations started. The following day the compression was carried out. Immediately before increasing the pressure, 0.03 mg/kg MK801 or saline was administered intravenously. Compression, with helium, proceeded at 0.33 ATA min^{-1} to 61 ATA. During this phase, 40 min hold periods were introduced at 31, 41 and 51 ATA, and then 61 ATA was held for 5 hr. Decompression then took place over the following 3 days, with the partial pressure of oxygen being raised to 0.7 bar. The baboon was then allowed 3 weeks for recovery, before repeating the procedure. Half of the animals were randomly allocated to receive MK801 on their first compression, half on the second.

Behavioural observations and scoring system

Continuous observations were made by two people throughout the compression, looking through separate portholes. They were each blind to both the treatment and precise pressure.

Table 1. High pressure neurological syndrome and tremor scores

Tremor:	Mild paw	1
	Mild limb	2
	Moderate limb	3
	Mild head	4
	Moderate head	5
	Severe limb	6
	Severe head	7
	Mild whole body	8
	Moderate whole body	9
	Severe whole body	10
Face tremor		4
Vomit		4
Myoclonus:	Limb +	1
	+ +	2
	Head +	2
	+ +	4
	Body +	3
	+ +	6

Pressures of onset were noted for tremor of the paw, limb and body, face tremor, myoclonus and vomiting. In addition, the degree of tremor the animal displayed was graded into mild, moderate and severe for each type of tremor, which was an agreed assessment between the two observers. From these observations, a tremor score was calculated as described in Table 1. The score was based on the fact that the tremor seen, as the pressure increased, was first manifested in the extremities and then spread centrally, while becoming more severe. In addition, a score for the high pressure neurological syndrome was also calculated from the tremor scores plus scores for myoclonus, face tremor and vomiting. To calculate a score for a given pressure, the maximum activity during the 10 min either side of this point was taken as the actual score. The criteria for this scoring system have been previously validated (Pearce *et al.*, 1989a).

Analysis of the EEG

Polygraph and tape recordings were made of the EEG and other signals, before and during the exposure period. Fourier analysis was carried out on 1 min of EEG data, using 6×10 sec epochs. Portions were analysed at 10 ATA intervals, at the beginning and ends of the 40 min hold periods and then every 1 hr during the hold at 61 ATA. Suitable portions of EEG were identified from the paper record and then located on tape. The recordings used were unfiltered and were acquired on-line, using an intelligent peripheral interface (CED 1401) and processed using associated software on an IBM PC (Cambridge Electronic Design). The analysis produced a frequency spectrum for each epoch of between 1 and 40 Hz and then calculated the mean amplitude of the frequency bands, delta (2–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–40 Hz).

To allow comparison between sections of EEG, taken at different times during the experiment, strict criteria were adopted in choosing the portions of EEG to analyse. These were:

1. The animal was still, with no movement artefacts on the trace. The EMG and respiration traces and behavioural remarks were then taken into consideration.
2. The animal was awake with its eyes open, though not staring. The EOG and EEG traces and behavioural remarks were used to assess this.
3. The amount of interference from eye movements was acceptable.
4. Episodic occurrences, such as short bursts of theta wave activity or spindling, were avoided.

Statistical methods

The Mann–Whitney U-test was used for the analysis of the pressure onset data. The existence of a carry-over effect was assessed by calculating the sum of the two measurements on each baboon and comparing these sums in animals receiving each treatment sequence. The existence of treatment and compression order, were tested by comparing the differences between the two measurements in baboons receiving each treatment sequence (Fleiss, 1986).

An analysis of variance was performed for the tremor and behavioural scores for the high pressure neurological syndrome. Pressures of 11 and 21 ATA were excluded from the analysis of variance, as the majority of scores were zero and it was not possible to satisfy the assumptions. Equality of residual variances was assessed using Schweder's method (Schweder, 1981) and Normality of residuals was tested using Shapiro and Wilk's W-test (Royston, 1982). A log (score $+1$) transformation was used. Three contrasts were included in the analysis of variance to test for (a) a linear trend during compression from 31 to 61 ATA, (b) an effect of the 40 min holds and (c) a linear trend during the 5 hr hold at 61 ATA.

An analysis of variance was also performed for the amplitude of the EEG for each wave and channel, separately. A log transformation was required to achieve equality of variances and Normality of residuals. The analysis was performed on the changes from baseline. Three contrasts were included in the analysis of variance to test for (a) a linear trend during compression from 11 to 61 ATA, (b) an effect of the 40 min holds and (c) a linear trend during the 5 hr hold at 61 ATA. There was a significant interaction between treatment and compression for channel 2, so the results presented are from a reanalysis, using the first compression only.

RESULTS

Pressure onset

Table 2 shows the median pressures and interquartile ranges at which signs of the high pressure

Table 2. Median and interquartile range (IQ) of pressure of onset (ATA) of signs of high pressure neurological syndrome in 8 baboons, with or without MK801

	Control		MK801		
Sign	Median	IQ range	Median	IQ range	*P* Value
Paw tremor	14	8 to 25	31	15 to 41	0.06
Limb tremor	19	15 to 30	29	22 to 40	0.06
Head tremor	49	35 to 54	60	25 to >61	0.9
Body tremor	61	59 to >61	>61	>61 to >61	0.03
Face tremor	60	36 to >61	>61	39 to >61	0.5
Limb myoclonus	>61	53 to >61	>61	61 to >61	0.9
Head myoclonus	>61	54 to >61	>61	>61 to >61	0.9
Body myoclonus	>61	54 to >61	>61	>61 to >61	0.14
Vomit	>61	57 to >61	>61	>61 to >61	0.11

P value is from the Mann–Whitney *U*-test, comparing control with MK801.

neurological syndrome appeared. As it was possible for the animals to control tremor of the paw and limb in the mild stages, once they first appeared, by holding on the solid objects, the initial onset pressures were used. Other types of tremor and myoclonus could occasionally occur for a brief period near the beginning of compression and then not appear continuously until a later pressure. In these cases onset pressures of continuous signs were noted.

In the Table, the results for all control and MK801-treated animals were grouped, irrespective of whether they come first or second in the randomised schedule of experiments. A pressure of >61 ATA is shown where a particular sign did not appear.

The pressures of onset of tremor of the paw, limb and body were significantly delayed after treatment with MK801 ($P = 0.06$, 0.06 and 0.03, respectively), although tremor of the head was not significantly affected. The onset pressures for face tremor, myoclonus and vomiting were also not significantly delayed by MK801. There was a significant effect of compression ($P = 0.03$) for head tremor, with the pressure of onset delayed on the second compression. There was significant carry-over ($P = 0.05$) for body tremor and vomiting, where pressure onsets were delayed and accelerated respectively, when the control treatment was given on the second compression.

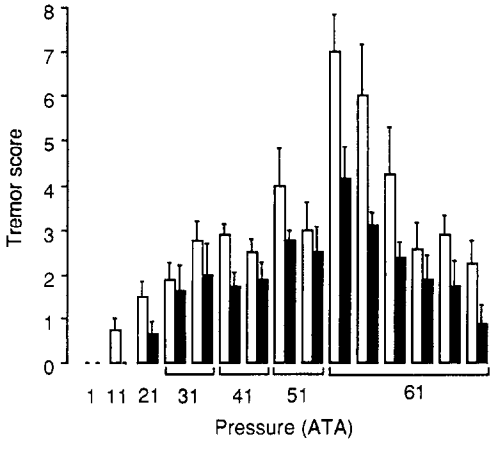

Fig. 1. Values for tremor score at increasing pressures. Scores are plotted for both the beginning and end of each 40 min period of constant pressure and for each hour, during the 5 hr hold at 61 ATA (indicated by ⌊⌋ on the *x*-axis). Open rectangles refer to control compressions; solid rectangles refer to compressions after treatment with MK801. (⊤ = SEM).

Tremor score

Figure 1 shows the mean tremor score at each interval of 10 ATA, including the hold periods. Scores for control and MK801-treated subjects are grouped together, irrespective of order of treatment. There was a significant increase in the score with increasing pressure ($P < 0.001$), with no significant decreases in score during the 40 min hold periods. The scores from the MK801-treated animals were significantly below the equivalent controls ($P < 0.001$). The mean level of tremor, at the highest pressure in the control animals, represented severe tremoring of both head and limb, whereas the mean level from the MK801-treated animals, represented just mild tremor of the head with moderate tremoring of the limb. The effect of MK801 was not dependent on the pressure attained ($P = 0.9$), that is, there were consistently significant differences between treatments at all pressures (quantitative data shown in Fig. 1). During the 5 hr hold at 61 ATA there was a significant trend in the reduction in scores ($P < 0.001$), which was parallel in both groups ($P = 0.8$).

The tremor scores tended to be slightly greater on the first compression ($P = 0.07$). There were no significant effects on the tremor score of which treatment came first.

High pressure neurological syndrome score

Figure 2 shows the mean high pressure neurological syndrome score at each interval of 10 ATA in the same manner as Figure 1 shows the tremor score. The score again increased with increasing pressure ($P < 0.001$), with no significant changes during the 40 min hold periods. The scores were significantly reduced ($P < 0.001$) by the presence of MK801 at all pressures. At 61 ATA, the mean decrease in the high pressure neurological syndrome score, due to MK801 was 5.5 which is greater than the effect of the drug on the tremor score (3.0). This was due to a reduction in the frequency of all three signs, face tremor,

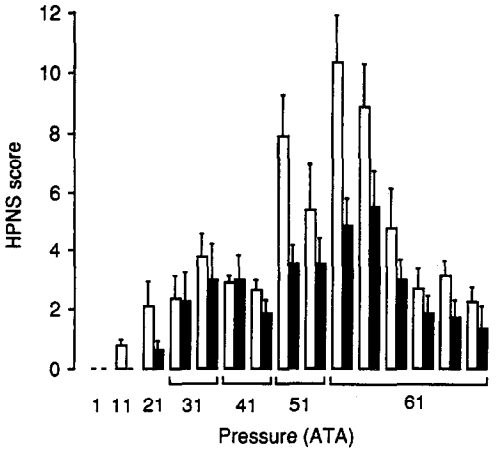

Fig. 2. Score for high pressure neurological syndrome (HPNS) values at increasing pressures (see Fig. 1 for legend).

myoclonus and vomiting, as well as tremor of the limb, head and body.

There was also a significant trend in the reduction in high pressure neurological syndrome scores ($P < 0.001$) over the 5 hr hold period at 61 ATA.

There were significantly different linear trends during increasing pressure, for the two compressions ($P = 0.04$), with the score tending to be less on the second compression at the lower pressures. As with the tremor score, there were no significant effects on the high pressure neurological syndrome score, of which treatment came first, i.e. there was no significant carryover of the effects of MK801.

Analysis of the EEG

Analysis of the EEG was carried out on all 3 channels at intervals of 10 ATA, including the beginnings and ends of the hold periods. In order to make comparisons, the amplitude of each frequency band, at each pressure interval, was log transformed and expressed as the change from the 1 ATA reading before increasing the pressure (Figs 3–5). Table 3 shows the P values, from the analysis of variance, assessing the changes occurring with pressure changes, hold, treatment and compression number.

Delta waves (2–4 Hz)

The amplitude of delta waves fell significantly ($P < 0.001$) as the pressure increased, in all three channels. In channel 1, the slope was greater on the control compression, than on the compression with MK801 ($P = 0.04$). There were no significant changes during the 40 min hold periods. There was a significant ($P = 0.008$) increase during the 5 hr hold at 61

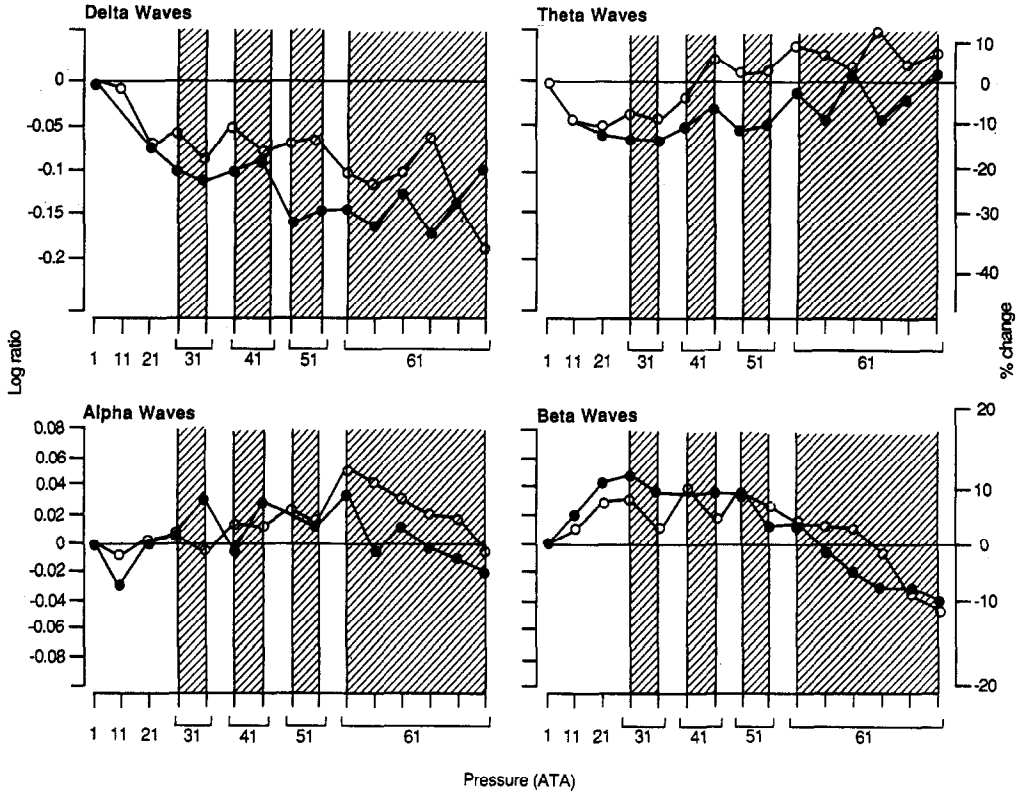

Fig. 3. Percentage change in the amplitude of EEG in channel 1 (frontal) for four wavebands (as described in the text), compared to initial baseline values at 1 ATA and plotted against increasing pressures. Data for control exposures (●) and exposures after treatment with MK801 (○) were analysed for both the beginning and end of each 40 min period at constant pressure and during the 5 hr hold at 61 ATA. Shaded areas indicate periods of hold. Statistical significances are detailed in the text.

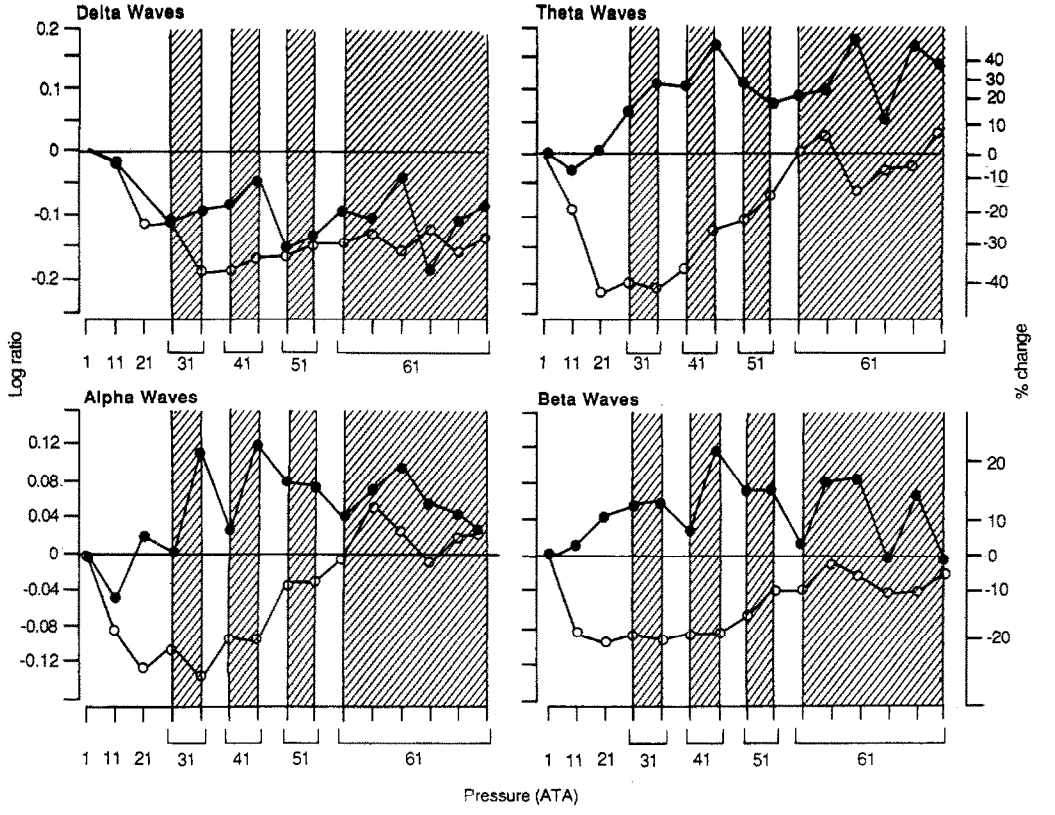

Fig. 4. As Figure 3, except the data are for channel 2 (parietal).

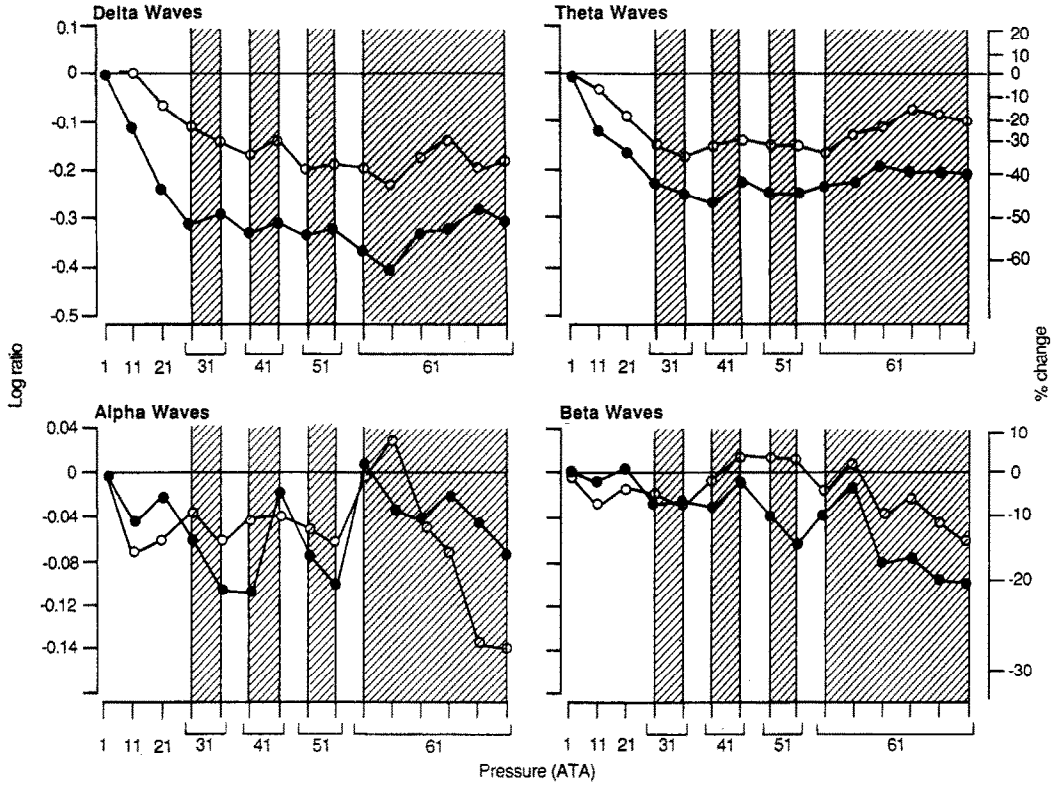

Fig. 5. As Figure 3, except the data are for channel 3 (occipital).

Table 3. Spectral analysis of EEG amplitudes: P values from analysis of variance

Source of variation	DF	Delta	Theta	Alpha	Beta
Channel 1 (frontal)					
Pressure: 11–61 ATA trend	1	<0.001*	<0.001*	0.004	0.4
40 min hold	1	0.5	0.7	0.7	0.008
61 ATA 5 hr hold trend	1	0.6*	0.8	0.006	<0.001**
Treatment	1	0.02	<0.001	0.2	0.9
Compression number	1	<0.001	0.9	0.4	0.002
Within-baboon error	161				
Channel 2 (parietal) (Analysis of Compression 1 only)					
Pressure: 11–61 ATA trend	1	<0.001	<0.001	<0.001	0.01
40 min hold	1	0.8	0.14	0.001***	0.04
61 ATA 5 hr hold trend	1	0.8	0.4	0.5	0.5
Treatment	1	0.6	0.13	0.3	0.13
Within-baboon error	77				
Channel 3 (occipital)					
Pressure: 11–61 ATA trend	1	<0.001	<0.001	0.3	0.9
40 min hold	1	0.9	0.6	0.9	0.7
61 ATA 5 hr hold trend	1	0.008	0.11	<0.001	0.02
Treatment	1	<0.001	<0.001	0.03	0.05
Compression number	1	0.4	<0.001	0.2	0.9
Within-baboon error	103				

*Significantly ($P < 0.05$) different linear trends for each treatment.
**Significantly different linear trends for combinations of compression number and treatment.
***Effect of 40 min hold varied significantly between treatments.

ATA in channel 3, while in channel 1 there was an increase on the control compression and a decrease on the compression with MK801 ($P = 0.03$). The reduction in amplitude was less severe after treatment with MK801 (channel 1, $P = 0.02$; channel 3, $P < 0.001$) (see Fig. 5 for channel 3 data). In channel 1, the reduction was more severe in those on the second compression, irrespective of treatment ($P < 0.001$). The amplitude had returned to pre-compression levels by the end of decompression in all 3 channels.

Theta waves (4–8 Hz)

The amplitude of theta waves fell during compression in channel 3 ($P < 0.001$) and increased in channel 2 ($P < 0.001$). In channel 1, the amplitude increased during compression after treatment with MK801 but not after control treatment ($P = 0.02$). In channel 1, amplitude of theta waves was greater after treatment with MK801 than in the controls ($P < 0.001$). In channel 3, the reduction was less after treatment with MK801 ($P < 0.001$) and also in those animals on the first compression ($P < 0.001$). There were no significant changes during either the 40 min or 5 hr hold periods. Pre-compression values had returned by the end of decompression.

Alpha waves (8–13 Hz)

The amplitude of alpha waves increased significantly during compression in channel 1 ($P = 0.004$) and channel 2 ($P < 0.001$). In channel 2, the effect of the 40 min hold periods varied with treatment ($P = 0.04$). The amplitude fell during the 5 hr hold period at 61 ATA in channels 1 ($P = 0.006$) and 3 ($P < 0.001$). In channel 3, the amplitude was significantly greater after treatment with MK801, compared with controls ($P = 0.03$). There were no significant effects due to number of compressions. Amplitude returned to pre-compression levels by the end of decompression.

Beta waves (< 13 Hz)

The amplitude of beta waves increased significantly during compression in channel 2 ($P = 0.01$). There was an increase during the 40 min holds in channel 2 ($P = 0.04$) and a significant decrease in channel 1 ($P = 0.008$). In channel 3, there was a significant reduction during the 5 hr hold at 61 ATA ($P = 0.02$), while in channel 1 there were significantly different linear trends for combinations of treatment and number of compressions ($P = 0.01$). The amplitude was significantly greater after treatment with MK801 in channel 3 ($P = 0.05$). In channel 1, the amplitude was significantly less on the second compression ($P = 0.002$).

Further analysis of the EEG

Plotting changes in waveband amplitude does not necessarily reflect all the changes occurring with pressure. For example, Figure 6 shows the mean percentage change of each frequency, from 1 to 61 ATA in channel 3. It can be seen that, in the control animals, a positive peak occurred at 9 Hz, i.e. in the alpha band, which was shifted 1 Hz to the left, into the theta band, in the MK801-treated animals. However, both the theta and alpha bands had negative values, as well as positive, thus reducing the overall change in that band. Delta waves in both groups were reduced by pressure and the beta wave band was mixed.

In channels 1 and 2 the positive peak for both groups was at 7 Hz, in the theta band, but, again, negative values were also present.

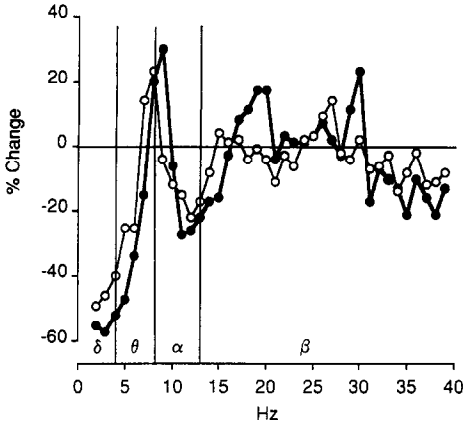

Fig. 6. The mean percentage change in amplitude of EEG for each frequency point, between 1 and 61 ATA in channel 3 (occipital). Control data (●); treatment with MK801 (○).

ADDITIONAL STUDIES

The observation, in one of the animals, that mild tremor of the head occurred earlier during compression after treatment with MK801 than would have been expected during a control compression, prompted some additional experiments, to observe the effects of a range of doses of MK801. The experiments were performed with the animals under normobaric conditions in air and also with the animals treated with greater pressures, at the compression rate previously described, but without incorporating the 40 min hold periods.

RESULTS

Table 4 summarises the behavioural remarks from the experiments where MK801 was given at 1 ATA air. In this context, "tranquillisation" refers to a preferred upright posture, although some effort was required to maintain this posture in those described as moderately tranquillised. Mild sedation refers to a preferred supine position, although on mild stimulation, movement was achieved with ease. Full sedation meant that the animal was supine for a 2–3 hr period and did not respond to stimulation. The animal which exhibited mild head tremor with the 0.03 mg/kg dose, was the same animal in which it was also observed earlier than expected after MK801, during exposure to pressure. Most of the animals showed disinterest in offered food and two, termed mildly tranquillised, were "quieter" than usual. The animal showing decreased anxiety allowed a much closer approach of humans than normal for that individual. The other animals were very tolerant of humans so, if this effect was present in them, it was not observable. The larger doses produced sedation, mild head tremor and occasional myoclonus.

Table 4. Effects of MK801 on behaviour at 1 ATA air

Dose (mg/kg)	No. of animals	Observation
0.03	4/6	↓ Appetite
	2/6	Mild tranquillisation
	1/6	Mild head tremor
0.06	1/2	↓Anxiety
	1/2	Moderate tranquillisation Mild head tremor Few arm/leg myoclonic jerks
0.12	1	Mild sedation
0.3	1	Full sedation Mild head tremor Arm/leg/head myoclonic jerks

Table 5. Summary of behavioural observations from additional compression experiments

Animal	Dose (mg/kg)	Pressure ATA	Observations
1	Control	71	Moderate body tremors, Face tremor, myoclonus
	0.6	81	As above
2	Control	61	Severe arm/leg tremor Moderate head tremor Myoclonus
	0.15	61	Mild tranquillisation Moderate head, arm and leg tremor
3	Control	81	Severe body tremor, Frequent myoclonus
	0.3	81	Full sedation Mild head tremor Frequent myoclonus

The larger doses of MK801 also provided protection against the severe aspects of high pressure neurological syndrome at the higher pressures. Table 5 summarises the behavioural observations at the highest pressures. At 0.06 mg/kg, MK801 did not reduce the severity of signs at 81 ATA but delayed the onset of that degree of severity by 10 ATA. The animal which received 0.15 mg/kg only achieved 61 ATA in its control compression but, at the same pressure after MK801, the signs were reduced to just mild arm tremors. Mild tranquillisation was, however, present. The animal treated with 0.3 mg/kg MK801 showed the same signs at 81 ATA as it did at 1 ATA; that is, full sedation, mild head tremor and myoclonus of the arm and leg. Fourier analysis of the EEG in this animal, in its control compression, showed a peak amplitude at 8 Hz in all 3 channels at 81 ATA. This was not present in the compression after MK801, nor in either of the analyses at 1 ATA.

Blood levels

The 0.5 ml plasma, available for assaying levels of MK801, did not prove sufficient and so a further experiment was carried out under the same conditions in the chamber, with larger blood samples (5 ml), obtained at the same times after administration of the same dose (0.03 mg/kg) of MK801, but without applying pressure. At 5 hr after administration, equivalent time to the 61 ATA sample, 1.6–1.7 ng/ml was detected. In other samples taken 24 hr, 72 hr and 7 days after administration, MK801 was not detectable.

DISCUSSION

The results clearly demonstrate that the non-competitive NMDA receptor antagonist, MK801, significantly ameliorated the severity of the high pressure neurological syndrome in the baboon. Onset pressures for tremors of the arm, leg and whole body were significantly delayed, on average by 10–17 ATA, although head tremor was not affected. This could have been due to some unknown excitatory side effects of MK801, producing head tremor itself, as was shown in two of the animals studied in the additional experiments. The appearance of face tremor, which is often regarded as facial myoclonus, was not affected by MK801. Other forms of myoclonus and vomiting, if they appeared, occurred late in compression and any effects on these parameters were not significant. The severity of the signs of the high pressure neurological syndrome, as defined in the tremor and scoring system for the high pressure neurological syndrome, was reduced at all the pressures studied. The raising of onset pressures meant that, at up to 20 ATA, most animals treated with MK801 showed no signs at all, whereas in the control compressions, mild tremors of the paw and limb had already started. These were similar to pressures at which a large proportion of commercial diving takes place and so, avoiding even mild tremors when working at these pressures, would be advantageous. Pressures, at which signs and symptoms occur, are very similar for man and the baboon (Rostain, 1980). At the higher pressures, the change in severity meant that the animal did not have to hold onto solid subjects to remain steady. Handling food was still possible and eating and drinking gave no apparent problem.

The factor, hold, was introduced to study both the effects of compression and of sustained pressure. The 40 min hold periods did not significantly reduce the severity of the signs and showed only minor changes in the EEG. The 5 hr period was, however, long enough to reduce the severity of signs, the reductions being parallel in both groups. Changes in the EEG also occurred during this time but were still minor. When compressing without the hold periods, initial observations suggested that, in the control animals, the signs at 61 ATA were less severe than when the hold periods were included, even though the compression rate was the same. Future investigations will further explore this aspect but it would appear that time, as well as rate of compression, is important in the establishment of the high pressure neurological syndrome in baboons.

Determination of levels of MK801 in plasma confirmed that the drug was being administered in significant quantities, but the level should not be regarded as absolute, because of the potential complicating effects of pressure. The non-detection of the parent compound, beyond 24 hr, is consistent with pharmacokinetic studies in other baboon models, where MK801 had a half-life in plasma of $1\frac{1}{2}$–2 hr (C. Brazell, personal communication).

The behavioural observations showed a distinct greater effect of MK801 over sodium valproate in ameliorating the severity of the high pressure neurological syndrome. In this previously reported study of baboons, treated with sodium valproate, carried out under the same conditions as the experiment reported here, none of the pressures of onset of signs, other than that for limb myoclonus, were delayed (Pearce *et al.*, 1989a). Analysis of the tremor score showed some reduction by sodium valproate but not enough to achieve statistical significance. The score for the high pressure neurological syndrome was reduced by sodium valproate but only achieved statistical significance at 51 and 61 ATA.

Thus, it would appear that, in the baboon, acute antagonism at the NMDA receptor site by MK801 (Wong *et al.*, 1986) provided greater inhibition of this excitatory phenomenon than the gradual increase in levels of inhibitory transmitter (γ-aminobutyric acid; GABA) (Chapman, Riley, Evans and Meldrum, 1982) and decrease in levels of aspartate (Schecter, Trainer and Grove, 1978), produced by chronic administration of sodium valproate. The non-competitive nature of the antagonism has the theoretical advantage over competitive blockade, produced by compounds such as 2-aminophosphonoheptanoic acid (Meldrum *et al.*, 1983), in that there is marked agonist dependence. A low physiological level of activation of NMDA receptors could still occur but if this was increased to pathological levels, extensive activation would bring about the block, whereas large concentrations of endogenous agonists may overcome the block produced by competitive antagonism (Kemp, Foster and Wong, 1987). Future experiments on the high pressure neurological syndrome and baboons will continue to explore this aspect, especially as competitive antagonists, which cross the blood–brain barrier and are orally active, are currently becoming available.

Fourier analysis of the EEG has produced complicated changes in all three channels studied. The most consistent changes occurring with pressure, have been reductions in the amplitude of delta waves which were less severe in the MK801-treated animals. Other variations have been shown by analysis of variance of the changes in amplitude of the 4 main frequency bands. However, as previously stated, this conventional form of analysis is limited, as it does not take account the possibility of a peak occurring in one part of the frequency band and a trough in another, thus potentially minimizing an overall change. The EEG analysis from animals compressed to 81 ATA showed larger peaks around 6–9 Hz, at the higher pressures (see Fig. 6). Thus, the prominent changes reported in human experiments (Bennett, 1982; Torok, 1984) may also be observed in the baboon but are probably related to a combination of time, pressure and compression rate.

There is considerable variation in the responses of different species to the actions of compounds possessing non-competitive antagonism at the NMDA receptor. The precise site of the anaesthetic action of ketamine is unknown, but among other suggestions, e.g. interactions with sodium channels (Allaoua and Chicheportiche, 1989), it is likely to involve antagonism of NMDA (Anis, Berry, Burton and Lodge, 1983). Ketamine acts as an anaesthetic in both rodents and primates and, at anaesthetic doses, was very effective against the high pressure neurological syndrome in rats (Green *et al.*, 1977). At sub-anaesthetic doses, in the rat, convulsions were delayed but tremor and myoclonus were unaffected (Wardley-Smith and Wann, 1989). In the baboon, however, at sub-anaesthetic doses, tremor, myoclonus and convulsions were all accelerated (J. A. S. Ross, personal communication). Phencyclidine is an anaesthetic in primates and in many other species, but the doses used in the rat to investigate actions on the high pressure neurological syndrome, which were effective doses in other seizure models, produced severe excitatory stereotyped behaviour (Wardley-Smith and Wann, 1989). The same authors used MK801 in rats, at the same doses as those used in the experiment reported here. The smaller dose (0.03 mg/kg) showed no effect whereas the larger dose (0.3 mg/kg) was accompanied by increased excitation on exposure to pressure. The sedative effects observed in this study on the baboon were not present in the rat, although the mild excitation, i.e. the head tremor, was possibly the result of the same molecular actions. Species variation has also been noted in other aspects of the actions of MK801, for example the "anti-conflict" effect, observed in rats, was not demonstrable in squirrel monkeys (Clineschmidt, Williams, Witoslawski, Bunting, Risley and Totaro, 1982). Caution would therefore be necessary in extrapolating data from primates to humans but, in view of these results, preliminary trials could be proposed. In its evaluation as an anti-epileptic drug, clinical trials suggested that there were no adverse toxic effects and only minor subjective side effects in a few patients, which were attributed to the central sympathomimetic properties (Troupin, Mendius, Chang and Risinger, 1986). However, the additional doses used in these experiments showed clear evidence of tranquillisation and sedation as well as significant amelioration of the signs of high pressure neurological syndrome signs, and so dose levels would need to be carefully monitored.

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