REFERENCES

Eberl, R., Dunky, A. (1983) Arzneimittel-Forsch. 33: 641–643

Ellis, C. N., Voorhees, J. J. (1983) J. Am. Acad. Dermatol. 8: 759–760

Levy, L. (1969) Life Sci. 8: 601–606

Petrick, T. J., Black, M. E. (1983) Arzneimittel-Forsch. 33: 619–680

Wada, Y., Etoh, Y., Ohira, A., Kimota, H., Koide, T. Ishihama, H., Mizushima, Y. (1982) J. Pharm. Pharmacol. 34: 467–468

Wax, J. (1978) Curr. Ther. Res. 23: 510–513

Wilkins, R. (1978) Ibid. 23: 581–584

Winthrop, G. J. (1982) N. Engl. J. Med. 307: 1578

J. Pharm. Pharmacol. 1987, 39: 59–62
Communicated May 29, 1986

Effects of microiontophoretic pentobarbitone on conditioned inhibitions mediated by GABA-A receptors in the cuneate nucleus of the rat in-vivo

F. ANDRES-TRELLES*, P. ORVIZ, *Facultad de Medicina, Departamento de Farmacologia y Terapeutica, Julian Claveria s/n, 33006 Oviedo, Spain*

We have studied the effects of microiontophoretic sodium pentobarbitone on the conditioned inhibition of the negative potential (N-wave) evoked in the cuneate nucleus of the rat by electrical stimulation (5 V, 0·2 ms, 0·5 Hz) of the ipsilateral forepaw. Five- or seven-barelled micropipettes were used, the tip being placed at a depth of 600–900 μm below the dorsal surface of the medulla oblongata. The conditioned inhibition was elicited by a previous identical stimulus. When the interval between the stimuli is shorter than about 40 ms (short duration) the inhibition is thought to be mediated by γ-aminobutyric acid (GABA), acting on GABA-A receptors. When it is longer (long duration conditioned inhibition) GABA-A receptors are not thought to be involved. Microiontophoretic sodium pentobarbitone potentiated both short (15 ms) and long (45 ms) duration conditioned inhibitions. The effect was current-dependent and appeared whether or not the first N-wave was depressed. Microiontophoretic application of (−)-bicuculline methiodide (a GABA-A antagonist) reduced the potentiation by pentobarbitone up to the basal inhibition when the interval between the stimuli was 45 ms or longer and to a greater extent when it was 30 ms or shorter. It seems likely that pentobarbitone prolongs the GABA-ergic mechanism which produces the short duration inhibition, making it visible with long stimulus intervals, superimposed upon the normal long duration conditioned inhibition which is not potentiated by local pentobarbitone.

Barbiturates potentiate inhibitions mediated by endogenous γ-aminobutyric acid (GABA) in the mammalian central nervous system, both in-vivo and in-vitro. Their mechanism of action appears to be the prolongation of the open time of the chloride channels associated with GABA-A receptors (Barker & Mathers 1981; Johnston & Willow 1982; Simmonds 1981). Ligand studies show that barbiturates inhibit the binding of [^{3}H]dihydropicrotoxinin, a non-competitive antagonist of GABA, to synaptic membranes (Olsen 1982; Ticku et al 1978). However, the actions of barbiturates in-vivo seem to be complex, judging from the variety of effects they cause (hypnotic, anaesthetic, anticonvulsant) (Enna 1981; Snodgrass 1983).

We describe here the effects of microiontophoretic sodium pentobarbitone, a hypnotic barbiturate, on a characterized and reproducible model of endogenous inhibition in-vivo: the inhibition by a previous stimulus (*conditioned* inhibition) of the negative potential (N-wave) evoked in the cuneate nucleus of the rat by electrical stimulation of the ipsilateral forepaw (Andersen et al 1970; Andres-Trelles et al 1976). When the interval between both stimuli is smaller than about 40 ms (*short duration* conditioned inhibition), the inhibition is due to GABA, since it is reduced by microiontophoretic GABA-A antagonists. When it is longer than 40 ms (*long duration*) the inhibition is resistant to GABA-A antagonists (Andres-Trelles et al 1976), as well as to antagonists of glycine, 5-hydroxytryptamine and histamine (unpublished observations). Nevertheless, the involvement of GABA in this long duration conditioned inhibition cannot be excluded, for it is now known that GABA receptors insensitive to (−)-bicuculline methiodide (GABA-B) are present in the cuneate nucleus of the rat (Orviz et al 1986).

Materials and methods

Male wistar rats (200–250 g) were anaesthetized with urethane (1·8 g kg^{-1} i.p.) or halothane (1–1·5% in 30% O_2 and 70% N_2O) and fixed in a stereotaxic frame. Two stainless steel electrodes placed in the centre pad of the paw and under the skin of the forelimb stimulated the forepaw at a rate of 0·5 Hz with supramaximal electric shocks (5 V), 0·2 ms wide. To study the conditioned

* Correspondence.

inhibitions we used standard stimulus intervals of 15 ms (short duration) and 45 ms (long duration).

The dorsal surface of the medulla oblongata was exposed and the potentials evoked in the ipsilateral cuneate nucleus were recorded via a silver–silver chloride electrode in one of the barrels, filled with 3 M NaCl, of a multibarrelled (five or seven compartments) borosilicate micropipette. Its DC impedance was lower than 4–6 MΩ. The tip of the micropipette (overall diameter 5–8 μm) was lowered into the nucleus to a depth of 600–900 μm, which, as previously reported, is the optimum position for recording the conditioned inhibition and the microiontophoretic effects of drugs (Andres-Trelles et al 1976; Orviz et al 1986). The indifferent electrode was placed under the skin of the head. The signal was led by a high impedance probe to a Grass P16 differential preamplifier from which the DC output was then led to a Neurolog System Digitimer amplifier and averager. Frequencies higher than 1 kHz were filtered out. The single sweeps and the averaged signal were displayed on a Tektronix oscilloscope. The displays were photographed either directly from the oscilloscope or after FM recording on a Hewlett-Packard 3964A recorder.

Solutions of sodium pentobarbitone (Abbott) 40 mM in distilled water, pH 9, and of (−)-bicuculline methiodide (Sigma) 5 mM in 150 mM NaCl, pH 3·5, were placed in the other barrels of the micropipette. These drugs were microiontophoretically applied into the cuneate nucleus by ejection currents lower than 100 nA, generated by a five channel Analog-Medical Systems Corp. microiontophoresis pump. Retaining currents of 25 nA were used to avoid passive diffusion. Balancing currents were automatically passed through one of the barrels, filled with 1 M NaCl.

Results

Microiontophoretic applications of sodium pentobarbitone significantly potentiated both short duration (15 ms stimulus interval) and long duration (45 ms interval) conditioned inhibitions (Figs 1, 2B). With higher ejection currents the effect was greater (Fig. 3). Overall, the amplitude of the first N-wave was not significantly affected with ejection currents up to 40 nA, but in particular experiments it was reduced and the reduction could be antagonized by (−)-bicuculline methiodide (Fig. 2A). In the experiments in which the amplitude of the first N-wave was not decreased (or was even slightly increased), the potentiation of the conditioned inhibitions by pentobarbitone was equally evident.

The GABA-A antagonist (−)-bicuculline methiodide, as expected, antagonized short but not long duration inhibition in control conditions (Fig. 1). However, the potentiation by pentobarbitone of both 15 and 45 ms inhibitions was diminished by (−)-bicuculline methiodide (Figs 1, 2B). After application of the antagonist in the presence of sodium pentobarbitone the degree of conditioned inhibition depended on the stimulus interval. When it was 45 ms or longer the amplitude of the second N-wave was comparable with the one recorded before the application of pentobarbi-

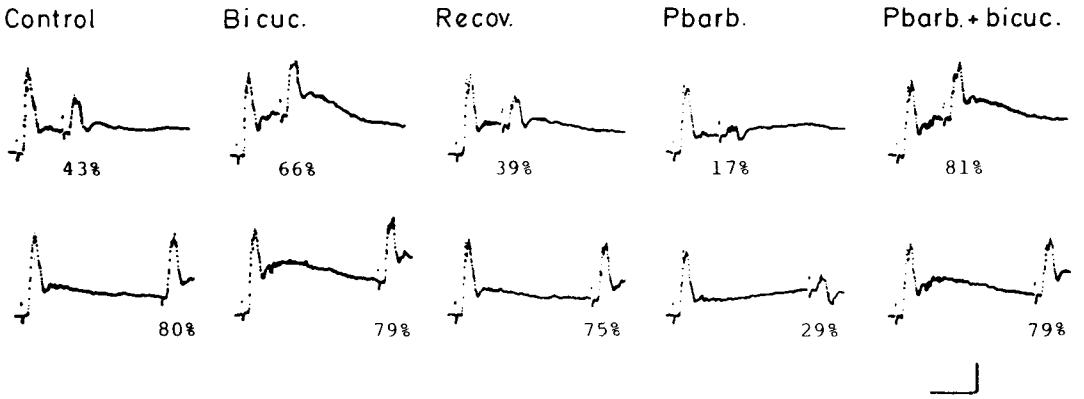

FIG. 1. Potentials evoked in the cuneate nucleus of an anaesthetized rat by two consecutive electric stimuli to the ipsilateral forepaw. Intervals between the stimuli are 15 ms (above) and 45 ms (below). The numbers show the amplitude of the second N-wave, expressed as a percentage of the first one, i.e. the smaller they are, the greater the conditioned inhibition is. Records are averages of eight consecutive single sweeps. Microiontophoretic (−)-bicuculline methiodide (Bicuc), 25 nA for 5 min, antagonizes the short but not the long duration conditioned inhibition. After recovering from bicuculline for 30 min, sodium pentobarbitone (Pbarb), 40 nA for 5 min, was microiontophoretically applied. It reduced the amplitude of the second N-wave. This potentiation of the conditioned inhibition was diminished by application of (−)-bicuculline methiodide (Pbarb + Bicuc), 25 nA for 5 min. Whereas the amplitude of the second N-wave was similar to the control when the interval was 45 ms, it was much greater when the interval was 15 ms. Vertical calibration bar = 250 μv negative upwards and horizontal bar = 15 ms.

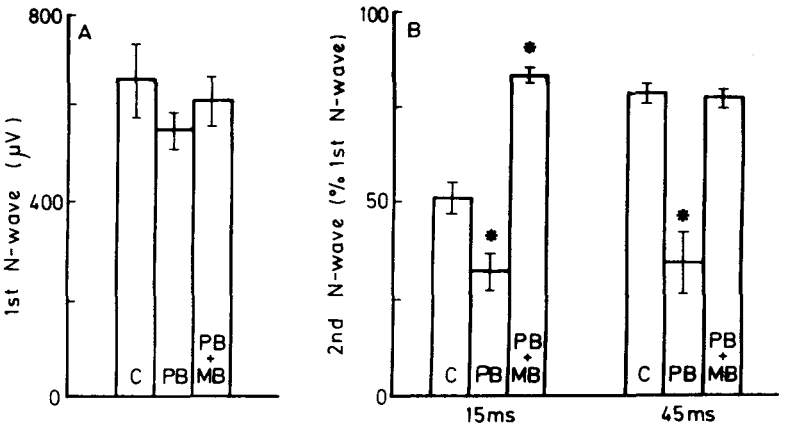

FIG. 2. A. Effect of microiontophoretic sodium pentobarbitone (PB), 40 nA for 5 min, on the amplitude of the first (i.e. non-inhibited) N-wave and influence on this effect of (−)-bicuculline methiodide (MB), 25 nA for 5 min. Vertical lines on each bar indicate the standard error of the mean (s.e.m.). C = Control. n ≥ 9. B. Effect of microiontophoretic sodium pentobarbitone (PB), 40 nA for 5 min, on the conditioned inhibition of the N-wave and antagonism by (−)-bicuculline methiodide (MB), 25 nA for 5 min. Intervals between the stimuli are 15 ms (left) and 45 ms (right). The histogram bars represent the amplitude of the second N-wave measured, as explained in Fig. 1, as a percentage of the first N-wave. As in A, vertical lines indicate s.e.m. C = control. * represents significant difference from the control ($P < 0{\cdot}05$, Student's *t*-test). n ≥ 9.

tone (control). On the contrary, when the interval between the stimuli was 30 ms or shorter the inhibition of the second N-wave was significantly smaller than the control (Figs 2B, 3).

Discussion

From our experiments it is clear that microiontophoretic sodium pentobarbitone potentiates the short duration conditioned inhibition of the N-wave, which seems to be mediated by activation of GABA-A receptors, as it is antagonized by (+)-bicuculline methochloride (Andres-Trelles et al 1976). The effect is dose-dependent and appears with doses (ejection currents) that do not affect the first N-wave. Hence, it does not seem to be due to direct depression of the evoked potential but to potentiation of an endogenous inhibitory mechanism. The same doses also potentiate the long duration conditioned inhibition. This is surprising, because in normal circumstances it is not sensitive to GABA-A antagonists. However, (−)-bicuculline methiodide also reduces this effect of pentobarbitone, as would be expected if it were caused by activated GABA-A receptors.

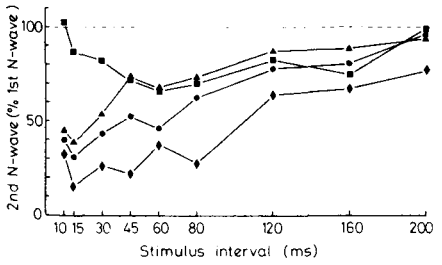

FIG. 3. Effect of different ejection currents of sodium pentobarbitone on the conditioned inhibition of the N-wave seen in an anaesthetized rat. The amplitude of the second N-wave is plotted, as a percentage of the first one, at each of several stimulus intervals (10 to 200 ms). ▲ Control. ● Sodium pentobarbitone, 20 nA for 5 min. ● Sodium pentobarbitone, 40 nA for 5 min. ■ (−)-Bicuculline methiodide, 25 nA for 5 min, in the presence of sodium pentobarbitone, 40 nA for 5 min.

A likely explanation of pentobarbitone potentiating the long duration inhibition seems to be that it prolongs the GABA-A-mediated mechanism which produces the short duration inhibition, making it visible with longer stimulus intervals. This is supported by the effect of (−)-bicuculline methiodide in the presence of sodium pentobarbitone at several stimulus intervals. When the interval is 15 ms, bicuculline antagonizes both the potentiation by pentobarbitone and the basal inhibition. When it is 45 ms or longer, bicuculline still antagonizes the effect of pentobarbitone but not the basal inhibition.

Our results show, therefore, the potentiation by local pentobarbitone of a GABA-A-mediated inhibition without obvious potentiation of other kinds of inhibition in the rat cuneate nucleus.

This work was supported by grant 82/224 from the Fondo de Investigaciones Sanitarias de la Seguridad Social. We thank Dr M. A. Simmonds for kindly reading a previous version of the manuscript.

REFERENCES

Andersen, P., Etholm, B., Gordon, G. (1970) J. Physiol. 210: 433–455

Andres-Trelles, F., Cowan, C. M., Simmonds, M. A. (1976) Ibid. 258: 173–181

Barker, J. L., Mathers, D. A. (1981) Trends Neurosci. 4: 10–13

Enna, S. J. (1981) in: Palmer, G. C. (ed.) Neuropharmacology of CNS and behavioral disorders. Academic Press Inc., New York, pp 507–537

Johnston, G. A. R., Willow, M. (1982) Trends Pharmacol. Sci. 3: 328–329

Olsen, R. W. (1982) Ann. Rev. Pharmacol. Toxicol. 22: 245–277

Orviz, P., Cecchini, B. G., Andres-Trelles, F. (1986) Rev. Esp. Fisiol. 42: 309–314

Simmonds, M. A. (1981) Br. J. Pharmacol. 73: 739–747

Snodgrass, R. (1983) in: Iversen, L. L., Iversen, S. D., Snyder, S. H. (eds) Biochemical studies of CNS receptors. Plenum Press, New York, pp 167–239

Ticku, M. K., Ban, M., Olsen, R. W. (1978) Mol. Pharmacol. 19: 319–402

J. Pharm. Pharmacol. 1987, 39: 62–63
Communicated July 10, 1986

Effects of dimethyl sulphoxide (DMSO) on aggregation of human blood platelets

B. LEHUU, P. B. CURTIS-PRIOR*, *Napp Research Centre, Science Park, Cambridge CB4 4GW, UK*

The effects were examined of the universal solvent dimethyl sulphoxide (DMSO) on human platelet aggregatory activity, in-vitro, of the endogenous mediators ADP, adrenaline, arachidonic acid, collagen and PAF-acether which are believed to play important roles in cardiovascular diseases in man. DMSO inhibited aggregation induced by all of the mediators in the order ADP > adrenaline = arachidonic acid = PAF-acether > collagen. Since DMSO is widely used as a solvent for drug substances, an awareness of its intrinsic activity in any such evaluations is essential.

Dimethyl sulphoxide (DMSO) was first synthesized over a century ago (Saytzeff 1867), though its useful solvent properties in relation to a variety of chemical substances have been recognized only within the last fifty years. It has, also, a wide spectrum of pharmacological activity of itself (David 1972; Haigler 1983; Brayton 1986) and has received renewed interest in the last few years as a therapeutic agent, particularly in North America. However, in view of reports linking DMSO to cataracts in laboratory animals (Rubin & Barnett 1967), and debate about its carcinogenic potential, some have cautioned its clinical use until adequate safety data become available (Savastano 1984).

Intravenously administered DMSO has been shown in-vivo to reduce the thrombotic response to surgical trauma (Dujovny et al 1983) in the rat and in a mouse model of pial arteriolar injury (Rosenblum & El-Sabban 1982) and to have anti-thrombotic effects when applied topically to rats (Gorog & Kovacs 1975). However, only few reports have appeared examining the effects of DMSO on platelet aggregation in-vitro, and of these there has been demonstrated an action against effects induced by ADP and adrenaline (Schiffer et al 1976) as well as thrombin (Holz & Davis 1972) and collagen (Shepherd et al 1984).

In view of the growing interest in discovering agents which beneficially modulate the roles of endogenous mediators in thrombosis and cardiovascular disease states, we have undertaken a systematic study of the effects of the universal solvent DMSO on aggregation of human platelets induced by ADP, adrenaline, arachidonic acid, collagen and PAF-acether.

Methods

Our studies of platelet aggregation were performed on citrated platelet-rich plasma (PRP). Blood was obtained via an antecubital vein from fasted healthy male adults who had not received any medication for at least one week before bleeding. PRP was prepared by centrifuging the citrated blood sample (9 volumes of blood to 1 volume of 3·8% aqueous sodium citrate) at 1500 rev min^{-1} for 15 min. The concentration of platelets in plasma was 300 000 ± 50 000 platelets μL^{-1}.

Aggregation experiments were conducted using a Chrono-log aggregometer model 550 (Coultronics), traces being recorded on an Omniscribe recorder. Aggregation was provoked by the following substances at the pre-determined concentrations indicated in parentheses: ADP (4 μM), adrenaline (1 μM), arachidonic acid (0·5 mM), collagen (10 μg mL^{-1}) and PAF-acether (1 μM). All the aggregating agents except PAF-acether, which were purchased from Sigma, were dissolved in physiological saline. PAF-acether (obtained from Bachem Corporation) was dissolved in 2·5% w/v aqueous bovine serum albumin (Fraction V).

Aggregation studies were conducted essentially as

* Correspondence.