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Effects of the anaesthetic 2,6-diisopropylphenol on synaptic transmission in the rat olfactory cortex slice

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1 The effects of the general anaesthetic 2,6-diisopropylphenol (DIP) on synaptic transmission and the actions of amino acid transmitter candidates have been investigated in rat olfactory cortex slices.

2 On electrical stimulation of the lateral olfactory tract (LOT), DIP (20 to 200 μM) increased the area of those surface field potentials which reflect γ-aminobutyric acid (GABA)-mediated transmission in a concentration-dependent manner in 6 out of 12 slices. In a series of conditioning experiments, DIP (50 μM) also potentiated GABA-mediated pre- and post-synaptic inhibition.

3 Perfusion of slices with DIP (50 μM) potentiated the reduction in the excitability of the terminals of the LOT produced by exogenous GABA in a picrotoxin-sensitive manner.

4 DIP (50 μM) markedly potentiated the surface depolarizations evoked by GABA, muscimol and 3-aminopropanesulphonic acid. The effect on the response to 3-aminopropanesulphonic acid was observed over a concentration range of DIP of 6.25 to 50 μM and was not blocked by the benzodiazepine receptor antagonist Ro 15-1788.

5 In slices in which GABA-mediated transmission was abolished by picrotoxin (25 μM), DIP (50 μM) had no significant effect on monosynaptically-evoked excitatory transmission but depressed the areas of those field potentials which reflect di-/polysynaptic excitations in a concentration-dependent manner (from between 1.6 and 6.25 to 50 μM).

6 In a series of conditioning experiments DIP (50 μM) abolished the increase in the excitability of the pyramidal cells evoked on stimulation of deep association fibres.

7 DIP (50 μM) had no significant effect on surface depolarizations evoked by N-methyl-D-aspartate, quisqualate and kainate or by the transmitter candidates L-glutamate and L-aspartate.

8 It is concluded that, at clinically relevant concentrations, DIP potentiates GABA-mediated transmission probably by an interaction with the GABA receptor complex and inhibits di-/polysynaptic excitations, possibly by inhibiting the release of excitatory transmitters.

Introduction

The intravenous anaesthetic 2,6-diisopropylphenol (DIP) has recently entered clinical use both for the induction and maintenance of anaesthesia. Although its pharmacokinetics have been investigated in some detail (Cockshott, 1985; Gepts *et al.*, 1985), little is known regarding the cellular actions of DIP. Results from an early study (Lodge & Anis, 1984) suggested that DIP might potentiate inhibition and attenuate excitations evoked in the spinal cord and, more recently, DIP has been shown to potentiate the actions of γ-aminobutyric acid (GABA) applied to bovine chromaffin cells in culture (Hales & Lambert, 1988). The aims of the present study were to assess the effects of DIP both on GABA-mediated synaptic inhibition and on mono- and polysynaptically-evoked excitations in the rat isolated olfactory cortex slice and, where possible, to identify its sites of action. Some of the results have been published in preliminary form (Collins & Anson, 1988).

Methods

Slices of olfactory cortex were prepared from freshly killed male rats (200 to 300 g, Wistar origin, University of Sheffield strain) using a bow cutter and perspex guide recessed to 500 μm. All slices were incubated for 2–3 h at room temperature in a salt

solution containing 0.2% v/v dimethyl sulphoxide (composition mM: NaCl 118.1, $NaHCO_3$ 25, D-glucose 11.1, $CaCl_2$ 2.5, KCl 2.1, $MgSO_4$ 1.1, KH_2PO_4 0.93), pH 7.3–7.4, continuously bubbled with 95% O_2 and 5% CO_2. Following incubation, slices were transferred to a perfusion apparatus (Pickles & Simmonds, 1976) and the undersurface perfused with solution at a rate of approximately 2–2.5 ml min^{-1}. The upper surface was exposed to water-saturated 95% O_2 and 5% CO_2 in order to prevent drying out.

Preparations were stimulated, using a tungsten metal microelectrode insulated with glass apart from 20 μm at the tip (Digitimer, Welwyn Garden City, U.K.) connected to a Grass S11 square wave stimulator with a constant current output photoelectric stimulus isolation unit (Grass Instrument Company, Quincy, U.S.A.), at a rate of one stimulus every 3 min. Evoked field potentials were recorded by means of a Ag/AgCl electrode located on the upper exposed surface of the slice, amplified with a d.c. amplifier and either captured in a Datalab DL1080 transient recorder (Datalab, Mitcham, U.K.) connected to a potentiometric plotter or recorded immediately with a Medelec u.v. system (Medelec, Woking, U.K.).

The positioning of the microelectrode enabled slices to be stimulated at one of three sites (see Collins & Howlett, 1988; Figure 1a and b). In the first, the preparation was mounted in the perfusion apparatus pial surface up and the tip of the microelectrode lowered 50 μm into the rostal lateral olfactory tract (LOT) which lies as a clearly visible white band of fibres on the surface of the olfactory cortex (Price & Sprich, 1975). The recording electrode was located 1 to 3 mm lateral to the LOT in the region of the prepyriform cortex (Heimer, 1968). Stimulus currents ranging from threshold to supramaximal were used as appropriate. In the second series of experiments, superficial association fibres were stimulated (Figure 1b). Slices which had been preincubated in solution containing picrotoxin (25 μM) were mounted pial surface up in the perfusion apparatus. The tip of the stimulating electrode was positioned toward the edge of the slice in the region of the prepyriform cortex and lowered 100 μm into the preparation. The recording electrode was sited 1.5 to 2 mm from the point of entry of the stimulating electrode in such a position that only the field potential known as the N'b'-wave was recorded. The position of the tip of the stimulating electrode was then adjusted in 20 μm steps until the amplitude of the evoked N'b'-wave was maximal and in order to prevent current spread to adjacent structures, submaximal stimulus currents were used (Collins & Howlett, 1988). Finally, the deep association fibres of other slices were stimulated. Following preincubation with picrotoxin

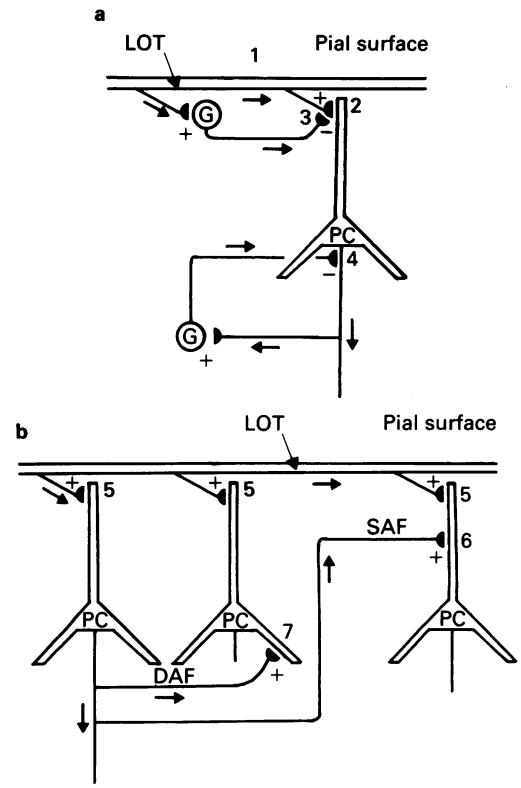

Figure 1 Diagrammatic representation of the inhibitory (−) and excitatory (+) inputs to the pyramidal cells (PC) of the olfactory cortex (modified from Haberly, 1985). In preparations in which synaptic inhibition was intact (a), stimulation of the lateral olfactory tract (LOT) evoked a series of surface field potentials consisting of (with increasing latency) a tract action potential (1), N-wave (2, a monosynaptically-evoked massed excitatory postsynaptic potential), late N-wave (3, a disynaptically-evoked depolarizing massed inhibitory postsynaptic potential evoked by γ-aminobutyric acid (GABA) released from inhibitory interneurones (G) acting on the terminals of the LOT) and I-wave (4, the surface correlate of GABA-mediated postsynaptic inhibition). In slices perfused with sufficient picrotoxin to abolish GABA-mediated inhibition (b), LOT stimulation evoked (with increasing latency) a tract action potential, N'a'-wave (5, the equivalent of the N-wave in control slices) and a N'b'- and P-wave, field potentials reflecting di-/polysynaptic excitation of PC by superficial association fibres (SAF, 6) and deep association fibres (DAF, 7) respectively. For references, see Haberly, 1985 and text.

(25 μM) slices were usually mounted cut surface up and the tip of the stimulating electrode positioned approximately 1.5 to 2 mm lateral to the LOT in the region of the prepyriform cortex. The tip of the electrode was lowered 100 μm into the upper cut surface

(i.e. nominally 400 μm deep to the pial surface). Again, the recording electrode was located between 1.5 and 2 mm from the site of entry of the tungsten microelectrode and a low stimulus intensity was used to limit the spread of current. Using this procedure, a surface field potential known as the inverted (because its polarity is the opposite of that evoked in slices mounted in the more usual configuration of pial surface up) P-wave is recorded in isolation (Collins & Howlett, 1988). The position of the tip of the stimulating electrode was then adjusted until the amplitude of the evoked potential was maximal.

When appropriate, evoked field potentials were quantified either by measuring their peak amplitudes, irrespective of latency (tract action potential, N- and N'a'-waves), or their areas (N'b'-wave, late N-/I-wave complex and inverted P-wave). Solutions of drugs were freshly prepared; DIP was mixed with dimethyl sulphoxide and diluted with the appropriate perfusion solution to give the required concentration of drug and a concentration of dimethyl sulphoxide of 0.2% v/v. Unless stated otherwise, solutions of DIP were applied dropwise to the exposed upper surface of slices as well as being present in the perfusion solution.

Studies of inhibition

Synaptically-evoked inhibition was assessed in slices mounted pial surface up by stimulation of the LOT using pairs of supramaximal stimuli (conditioning intervals of between 0.02 and 90 s). Presynaptic inhibition was measured by expressing the amplitude of the field potential known as the N-wave (Figure 1a) evoked by the second (test) stimulus as a percentage of that evoked by the first (conditioning) pulse. The reduction in the amplitude of the test response over conditioning intervals of approximately 0.05 to 0.2 s is thought to reflect a dimunution in transmitter release from the LOT fibre terminals mediated by GABA released by the conditioning stimulus (Pickles & Simmonds, 1976). When postsynaptic inhibition was monitored, the position of the recording electrode was adjusted so that a clear population spike was superimposed on the evoked N-wave (Pickles & Simmonds, 1978). The population spike represents the summed action potentials of monosynaptically-activated pyramidal cells (Richards & Sercombe, 1968): an increase in latency reflects an increase in GABA-mediated recurrent inhibition triggered by the conditioning pulse (Pickles & Simmonds, 1978).

Excitability testing of lateral olfactory tract terminals

The effects of DIP on the reduction in the excitability of the terminals and pre-terminal axons of the LOT fibres (Cain & Simmonds, 1982) was also assessed. The cortex adjacent and sub-adjacent to the rostral LOT of slices was dissected away and following preincubation for 2–3 h, slices were mounted cut surface up in the perfusion apparatus. The rostral end of the LOT was drawn into a suction electrode which was then raised above the level of the slice. A glass-insulated tungsten microelectrode for stimulation was located either vertically above or up to 1 mm lateral to the LOT and lowered in 20 μm steps into the cut surface of the slice. Stimulation was at a rate of 0.25 Hz, 50 μs pulse width, submaximal current. As the stimulating electrode was progressively lowered, a short duration (approximately 10 ms) positive-going potential was recorded by the suction electrode. Its sensitivity to tetrodotoxin (2.25 μM) but not to Cd^{2+} (1 mM) suggested that it was an antidromically propagating compound action potential (see Cain & Simmonds, 1982). When assessing terminal excitability, it was essential to make certain that the site of stimulation was the terminals and pre-terminal axons but not the myelinated fibres of the LOT. This was routinely achieved by ensuring that (i) the latency to peak of the potential (2.5 to 4 ms) was greater than that evoked when the electrode was lowered into the LOT itself (1.2 to 2.5 ms); (ii) the potential was reduced in amplitude by GABA applied to the upper cut surface of the slice (Cain & Simmonds, 1982); and (iii) the optimal depth for stimulation was between 300 and 400 μm from the myelinated fibres lying on the pial surface (Price & Sprich, 1975). The stimulus current was adjusted to evoke potentials of between 40 and 50% of maximum. Pairs of stimuli were averaged using a Neurolog NL 750 averager (Digitimer, Welwyn Garden City, U.K.) and every fifteenth signal plotted using a potentiometric plotter. Cumulative dose-response relationships to GABA were constructed by applying each concentration dropwise to the upper cut surface of the slice for a sufficient time period to achieve a plateau effect (3 to 5 min). Each preparation was used for one dose-response study and then discarded. The effect of GABA was quantified by calculating the percentage reduction in amplitude of the compound action potential. In those experiments in which the effects of picrotoxin or DIP on the actions of GABA were monitored, the drugs were present in the perfusion solution as well as being applied to the exposed cut surface of the slice. The results were evaluated with a two factor analysis of variance followed by Dunnett's test for the comparison of drug treatments.

Studies of excitation

A series of conditioning experiments was carried out in order to determine any functional consequence of

the actions of DIP on di-/polysynaptically-evoked excitations. In principle, the effect of conditioning pulses applied to deep association fibres (see Figure 1b) on the test response evoked on submaximal stimulation of the LOT was assessed. Olfactory cortex slices which had been preincubated in solution containing picrotoxin (25 μM) were mounted pial surface up in the perfusion apparatus. One tungsten microelectrode was inserted 50 μm into the surface of the LOT (see above). A second microelectrode was positioned approximately 2 mm lateral to the LOT in the prepyriform cortex and the tip lowered 400 μm into the slice. The Ag/AgCl recording electrode was sited on the pial surface in such a position that it could record the potentials evoked by both stimulating microelectrodes. Care was taken to ensure that the P-wave evoked on stimulation of the deep association fibre was uncontaminated by any other field potentials and that the test response (N'a'-wave) evoked on LOT stimulation was uncontaminated by population spikes. The latency to peak of the N'a'-wave was plotted against the conditioning interval (20 to 2000 ms).

Measurement of amino acid-evoked depolarizations

In some experiments, amino acid-evoked changes in the d.c. potential across slices were measured using the technique originally described by Brown & Galvan (1979) as modified by Collins & Brown (1986) and Collins & Surtees (1986). Briefly, extracellular electrodes recorded the potential difference across an olfactory cortex slice which had been preincubated and perfused with picrotoxin (25 μM). If a drug which depolarizes neurones is applied to one surface of the slice, that surface will become negative with respect to the other surface. Two series of experiments were carried out. In the first, the effect of DIP (50 μM) on submaximal responses evoked by single concentrations of the excitatory amino acids L-aspartate (10 mM), L-glutamate (10 mM), kainate (0.1 mM), N-methyl-D-aspartate (0.1 mM) and quisqualate (0.2 mM) was assessed. In the second series of experiments, dose-response curves to GABA (0.05 to 1 mM), 3-aminopropanesulphonic acid (0.005 to 0.05 mM) and muscimol (0.001 to 0.1 mM) were constructed both in the absence and presence of DIP (50 μM). It should be noted that the responses evoked by excitatory amino acids in the preparation show the expected pharmacology to both selective and non-selective excitatory amino acid receptor antagonists (Collins & Brown, 1986).

Drugs

DIP was purchased from the Aldrich Chemical Company and purified by fractional distillation *in vacuo*. All amino acids plus picrotoxin were from the Sigma Chemical Company. Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a][1,4]benzodiazepine-3-carboxylate) was a gift from Roche.

Results

Effects of 2,6-diisopropylphenol on inhibition

If an olfactory cortex slice is perfused pial surface up and the recording electrode sited on the exposed surface in the region of the prepyriform cortex, supramaximal stimulation of the LOT evokes a series of potentials consisting of (with increasing latency) a tract action potential (not shown) and three negative-going potentials, the N-, late N- and I-wave (Figures 1a and 2). Application of DIP (20

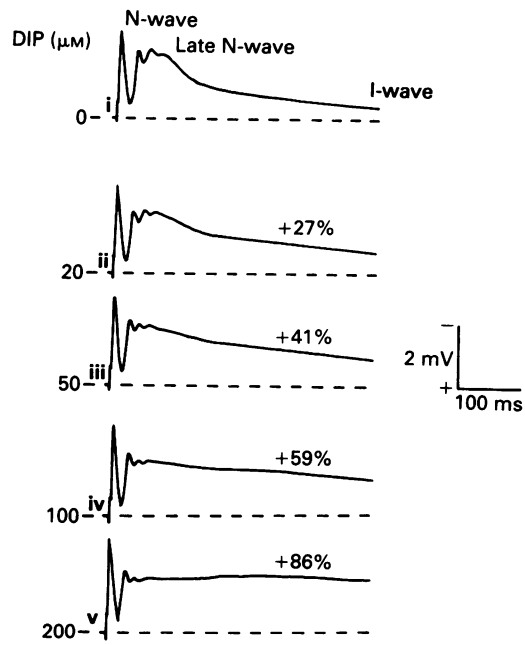

Figure 2 Effect of 2,6-diisopropylphenol (DIP) on evoked field potentials. The panels show the surface field potentials (retouched) evoked on supramaximal stimulation of the lateral olfactory tract (LOT) recorded in the absence (i) and on the cumulative application of DIP (ii–v) to a single slice mounted pial surface up. Each concentration of the drug was applied for 15 min before recording the evoked potentials. The values in (ii–v) are increases in the areas of the late N-/I-wave complexes expressed as percentages of that evoked in the absence of DIP. No recovery was attempted. Note that DIP has no significant effect on the N-wave. Similar results were seen in 5 other slices (see text for further details).

to 200 μM) had no effect on the latency or amplitude of the action potential or N-wave. However, in 6 out of 12 preparations, DIP caused a concentration-dependent increase in the area of the late N-/I-wave complex. In 5 other slices, the effect was independent of drug concentration whilst there was a reduction in the area of the late N-/I-wave in one preparation. These effects were partially reversible on application of drug-free solution for 1 h (not shown). The variability of the effects of DIP is discussed later. The late N- and I-waves reflect GABA-mediated pre- and post-synaptic inhibition, respectively (Pickles & Simmonds, 1976; 1978) and so the possible effects of DIP on evoked inhibition were investigated.

Presynaptic inhibition was monitored by measuring the amplitudes of the N-waves evoked on supramaximal stimulation of the LOT by pairs of pulses (see Methods section). In the control experiment illustrated in Figure 3a, the amplitude of the N-wave evoked by the second (test) stimulus was less than that evoked by the first (conditioning) pulse over conditioning intervals of between approximately 0.05 and 0.2 s. Application of DIP (50 μM) increased both the reduction in amplitude of the test response and the range of conditioning intervals over which the phenomenon was observed. The effect of DIP was partially reversible (Figure 3a).

Postsynaptic inhibition was assessed by measuring the latency of the population spike evoked by the second of a stimulus pair. Application of DIP (50 μM) reliably caused a small and reversible increase in the latency of the population spike (Figure 3b).

Effect of 2,6-diisopropylphenol on the actions of exogenous GABA

Two series of experiments were carried out to determine the effects of DIP on the actions of exogenous GABA. In the first, the terminals and preterminal axons of the LOT fibres were stimulated submaximally using a focally sited microelectrode and the compound action potentials propagating antidromically in the LOT recorded with a suction electrode. Application of GABA (0.1 to 1 mM) reduced the amplitude of the compound action potential (Figure 4; see also Cain & Simmonds, 1982). Application of DIP (50 μM) potentiated the effects of GABA in a picrotoxin-sensitive manner, but in concentrations up to 200 μM did not mimic the actions of GABA (not shown).

In the second series of experiments, pial-surface depolarizations evoked by GABA, 3-aminopropanesulphonic acid and muscimol were recorded. Application of DIP (50 μM) potentiated the responses evoked by all three agonists (Figure 5a). The log dose-response curves to the agonists were

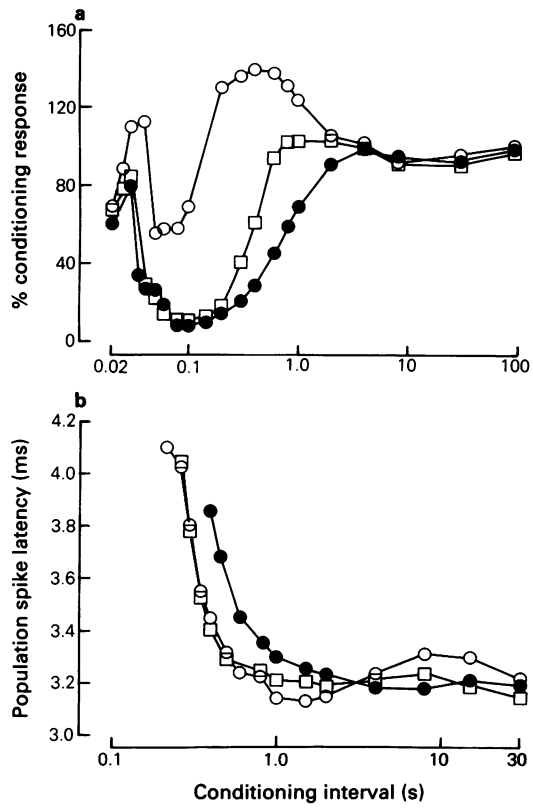

Figure 3 Effect of 2,6-diisopropylphenol (DIP) on evoked inhibition. Each of the two panels illustrates the results of a single experiment carried out using different preparations. The general procedure was to stimulate the lateral olfactory tract (LOT) of the slice using pairs of supramaximal stimuli before (○) and during application of DIP (50 μM) for 15 min (●) and following perfusion with drug-free solution for 90 min (□). (a) Presynaptic inhibition was monitored by calculating the ratio of the amplitude of the N-wave evoked by the second (test) stimulus as a percentage of that evoked by the first (conditioning) pulse. Note the dramatic potentiation in the reduction of the ratio over conditioning intervals of between approximately 0.05 and 0.2 s. (b) Postsynaptic inhibition was assessed by measuring the latency of the population spike evoked by the test stimulus. Note the upward shift in the curve to the right produced by DIP (50 μM). Each experiment was repeated on four other slices with similar results.

shifted to the left (Figure 5b). With GABA the shift was parallel, whereas with the other agonists there was an increase in the slope of the log dose-response curve. The potentiation of the evoked responses to 3-aminopropanesulphonic acid by DIP was concentration-dependent with a threshold concentration of less than 6.25 μM (Figure 6a; GABA and

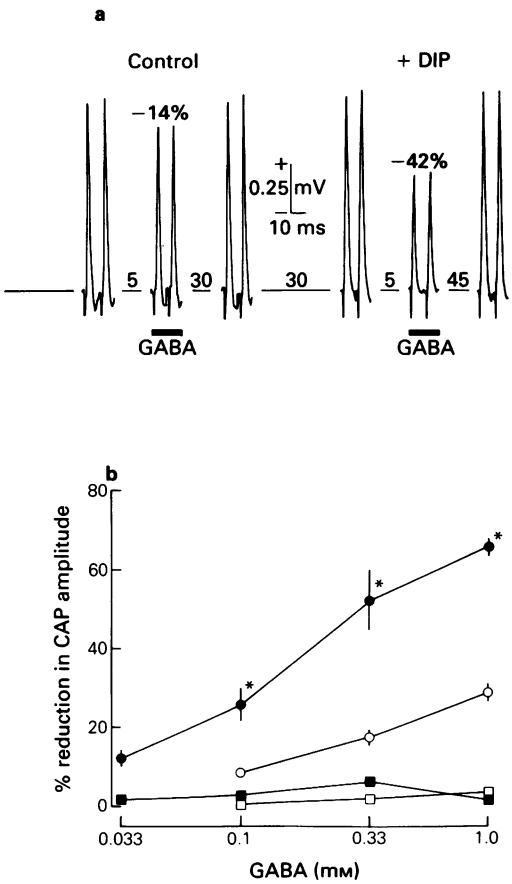

Figure 4 Potentiation of the depressant effects of γ-aminobutyric acid (GABA) on terminal excitability by 50 μM 2,6-diisopropylphenol (DIP). Antidromically propagating compound action potentials (CAPs) evoked by submaximal stimulation of the lateral olfactory tract (LOT) terminals were recorded in the LOT fibres using a suction electrode (see Methods). (a) Results from a single experiment. Each peak is the average of 2 CAPs and there is a 1 min delay between the potentials comprising each pair. The solid horizontal lines represent the recording baseline and the adjacent numbers are the delays between each pair of recordings in min. Application of GABA (0.33 mM) for 5 min reduced the mean amplitudes of the compound action potentials by 14%. Following perfusion with DIP for 15 min, the same concentration of GABA now reduced the amplitude by 42%. Note that DIP alone was without effect on the compound action potentials. (b) Cumulative dose-response curves of the effects of GABA alone (○), GABA plus DIP (●), GABA plus picrotoxin (0.1 mM; □) and GABA plus picrotoxin plus DIP (■) on the amplitudes of the evoked CAPs. Each point is the mean of 5 observations and s.e.mean, when greater than the symbols, is indicated by the vertical lines. * Indicates a significant difference (Dunnett's test) when compared with the effects of GABA alone ($P < 0.01$).

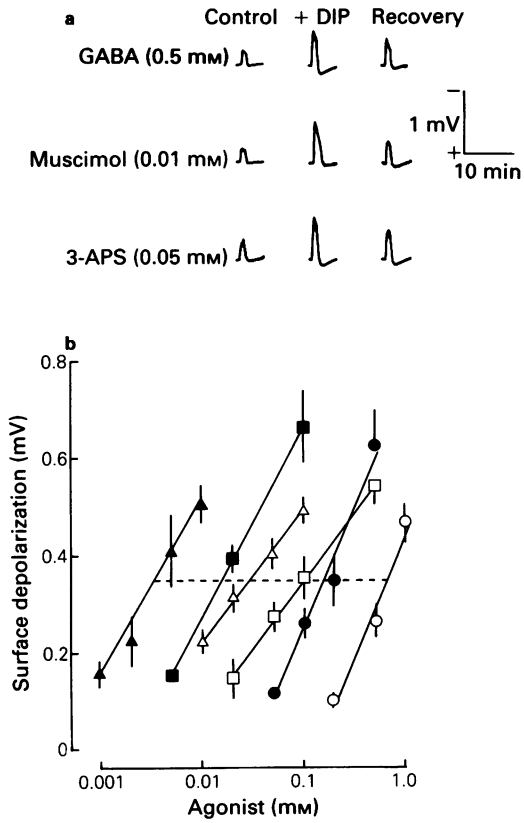

Figure 5 Potentiation by 50 μM 2,6-diisopropylphenol (DIP) of the depolarizations evoked by γ-aminobutyric acid (GABA), muscimol and 3-aminopropanesulphonic acid (3-APS). (a) Results from a single experiment. DIP, when perfused onto the pial surface of the slice for 15 min, potentiated the responses to all three agonists. Partial recovery is seen after perfusion with drug-free solution for 90 min. Note that DIP alone (up to 200 μM) did not mimic the action of GABA. (b) Dose-response curves to agonists alone (open symbols) and in the presence of DIP (solid symbols); GABA (○, ●); 3-APS (□, ■); muscimol (△, ▲). Each point is the mean of either 4 or 6 observations, the s.e.means being indicated by the vertical lines. Dose-ratios were measured for each pair of dose-response curves at a depolarization of 0.35 mV (dashed horizontal line) and the means ± s.e.means calculated: GABA, 0.258 ± 0.037 ($n = 6$); 3-APS, 0.150 ± 0.022 ($n = 4$); muscimol, 0.114 ± 0.009 ($n = 4$).

muscimol not tested). Finally, it was shown that although the benzodiazepine receptor antagonist Ro 15-1788 blocked the potentiation of the response to 3-aminopropanesulphonic acid by medazepam (5 μM), it did not antagonize the potentiating action of 50 μM DIP (see Figure 6b).

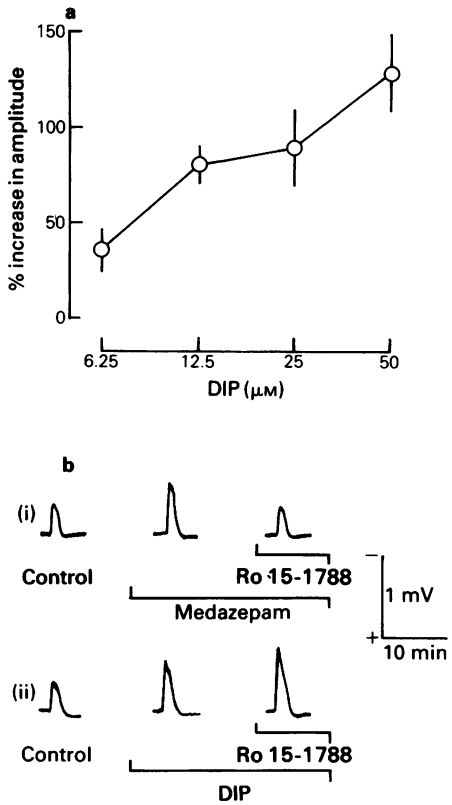

Figure 6 Potentiation of responses evoked by 3-aminopropanesulphonic acid (3-APS, 0.05 mM) by 2,6-diisopropylphenol (DIP) and medazepam (5 μM). (a) Dose-response curve to DIP. Each point is the mean of 6 observations and the s.e.means are indicated by the vertical bars. (b) Effect of Ro 15-1788 (5 μM) on the potentiation by medazepam (i) and DIP (50 μM, ii) of 3-APS evoked depolarizations. Each set of three responses was from a different slice. Control, 3-APS for 1 min alone. Medazepam or DIP was then perfused onto the slice for 30 min and the dose of 3-APS reapplied before and 15 min after the addition of Ro 15-1788. Note that the response to 3-APS in the presence of DIP is apparently potentiated by Ro 15-1788. Similar results were seen in 4 other experiments.

Effects of 2,6-diisopropylphenol on excitation

In slices, mounted pial surface up and preincubated and perfused with solution containing sufficient picrotoxin to abolish GABA-mediated transmission, stimulation of the LOT evokes a surface negative potential known as the N'a'-wave (equivalent to the N-wave evoked in the absence of picrotoxin) followed by the longer latency N'b'- and P-waves (not shown), two potentials which reflect di-/polysynaptic excitation of pyramidal cells (Gilbey & Wooster, 1979; Collins *et al.*, 1982; see Figure 1b). Application of DIP (25 to 200 μM) had no significant effect on either the N-wave (Figure 2) or N'a'-wave (not shown) but appeared to reduce the amplitude of the N'b'-wave and alter the configuration of the P-wave (not shown). In order to identify precisely the effects of DIP on polysynaptically-evoked excitations, focal stimulation of the two association fibre systems (Figure 1b) was achieved using the procedures described by Collins & Howlett (1988). Submaximal stimulation of superficial association fibres evoked the equivalent of the N'b'-wave uncontaminated by other potentials (Figure 7a). DIP (1.56 to 50 μM) caused a concentration-dependent reduction in the

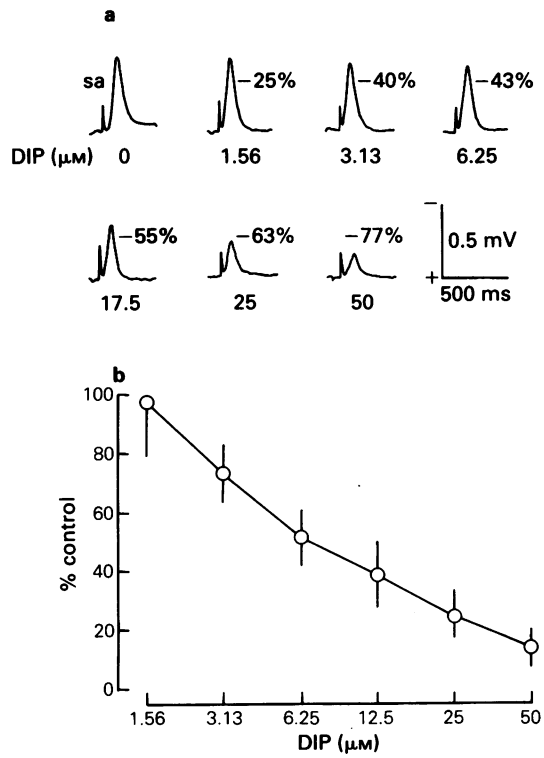

Figure 7 Effects of 2,6-diisopropylphenol (DIP) on the N'b'-wave evoked on submaximal stimulation of superficial association fibres. (a) Results from a single slice mounted pial surface up, and perfused with solution containing picrotoxin (25 μM). Each panel shows the potential (retouched) recorded following application by DIP for 30 min. The relevant concentration is indicated under each response and the value adjacent to each peak is the reduction in area of the potential expressed as a percentage of that evoked in the absence of DIP. sa, stimulus artefact. (b) Cumulative dose-response curve for DIP. Each point is the mean of 6 values and the s.e.mean is indicated by the vertical lines.

area of the potential (Figure 7b). In experiments in which slices were mounted cut surface up, focal stimulation of the deep association fibres (Figure 1b) evoked an inverted surface P-wave complex uncontaminated by other potentials (Figure 8). Concentrations of DIP between 3.125 and 6.25 μM transiently increased the area of this potential in 3 out of 4 slices. Higher concentrations abolished the inverted P-wave although this could be overcome by an increase in the stimulus current (Figure 8).

The possible functional significance of the reduction in the areas of the N'b'- and P-waves by DIP was investigated in a series of conditioning experiments. Slices which had been preincubated in the presence of picrotoxin were mounted pial surface up (see Methods section). Conditioning pulses were applied to the deep association fibres by a microelectrode located 400 μm from the pial surface and test pulses to the LOT: the consequent surface field potentials were recorded in the usual manner (Figure 9a). Over conditioning intervals of approximately 20 to 200 ms, the latency to peak of the N'a'-wave evoked by the test pulse was reduced, indicating an increase in pyramidal cell excitability. The extent of the reduction was inversely related to the intensity of the test stimulus (not shown) and was abolished by DIP (50 μM) in a partially reversible manner (Figure 9b).

Effect of 2,6-diisopropylphenol on the actions of exogenous excitatory amino acids

Perfusion of slices with DIP (5 to 200 μM) had no effects on the depolarizations evoked by single submaximal concentrations of L-glutamate, L-aspartate, N-methyl-D-aspartate, kainate or quisqualate (not shown).

Discussion

The aim of the present study was to determine the effects of clinically-relevant concentrations of the anaesthetic DIP on synaptic transmission in the

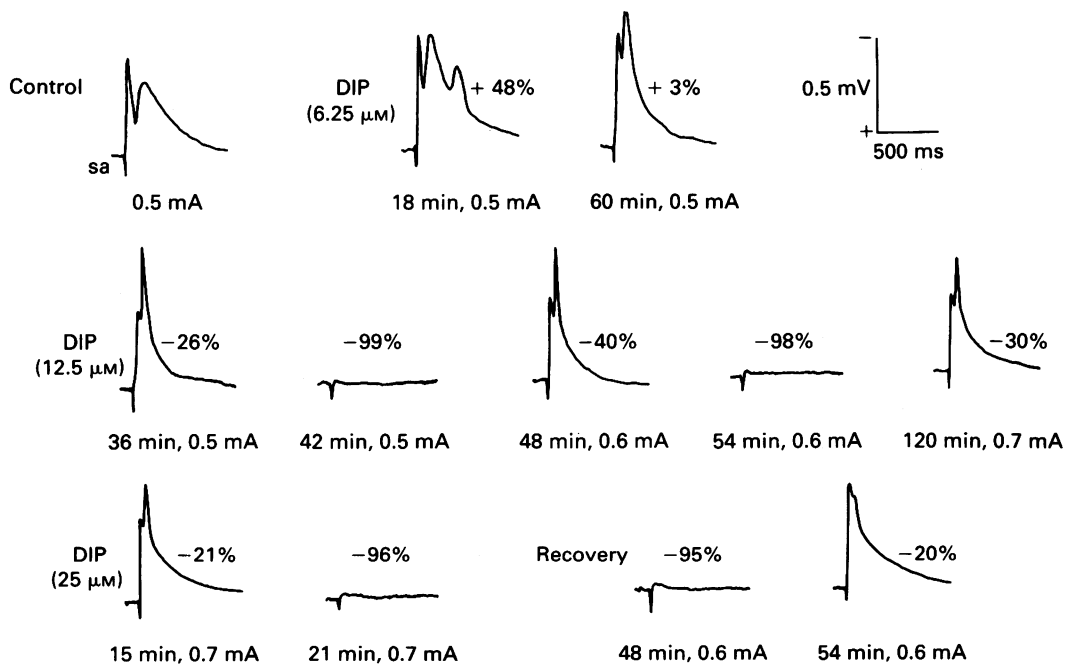

Figure 8 Effect of 2,6-diisopropylphenol (DIP) on the inverted P-wave complex evoked on stimulation of deep association fibres in a single slice mounted cut surface up, and perfused with solution containing picrotoxin (25 μM). The responses illustrated are the potentials (retouched) evoked using the stimulus currents indicated and are shown in sequence. The value adjacent to each peak is the change in area expressed as a percentage of that evoked in the absence of DIP (control). The slice was perfused with increasing concentrations of DIP in a cumulative manner for the times indicated followed by drug-free solution for recovery. In the absence of DIP, a stimulus current of 0.5 mA was threshold and and the potential was all-or-none in nature (Collins & Howlett, 1988). Note that 6.25 μM DIP transiently increased the area of the potential and that higher drug concentrations abolished the potential, an effect which was overcome by an increase in the stimulus current. Similar results were seen in 3 other slices.

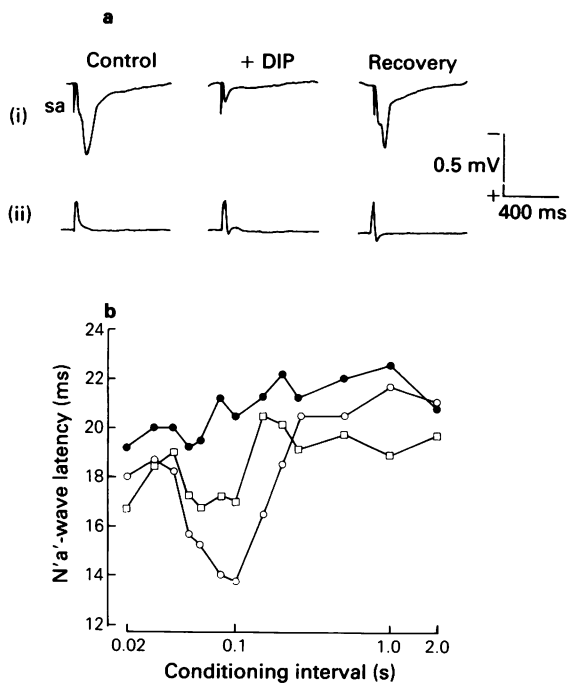

Figure 9 2,6-Diisopropylphenol (DIP; 50 μM) abolishes the increase in pyramidal cell excitability evoked by submaximal stimulation of deep association fibres. The results illustrated in both panels are from the same single experiment using a slice mounted pial surface up, and perfused with solution containing picrotoxin (25 μM). (a) Surface potentials (retouched) recorded on submaximal stimulation of deep association fibres (conditioning pulse, i) or lateral olfactory tract (test pulse, ii) evoked before (control), following 60 min perfusion with DIP and after perfusion with drug-free solution for 90 min (recovery). sa, stimulus-artefact, (b) latency to peak of N'a'-wave evoked by the test stimulus as a function of the conditioning interval. (○) Control, (●) DIP (60 min), (□) partial recovery after perfusion with drug-free solution for 90 min. Similar results were seen in 4 other experiments.

olfactory cortex. The experimental approach was to identify the actions of the drug on the field potential correlates of both excitatory and inhibitory transmission and then to assess the possible functional significance of any such effects. Finally, the actions of DIP on the response evoked by some of the transmitter candidates of the olfactory cortex were measured in an attempt to identify whether the sites of action of DIP were pre- and/or post-synaptic. One potential difficulty in the interpretation of the results was the use of a high concentration of dimethyl sulphoxide (approximately 25 mM) to dissolve the anaesthetic. Although the pharmacological actions of dimethyl sulphoxide were not studied in detail, application of the solvent (0.2% v/v) to preparations ($n = 6$, not shown) had no consistent effects on any of the field potentials. Moreover, as dimethyl sulphoxide (0.2% v/v) was present both during preincubation and continuously throughout perfusion of the slices, there is no possibility that the pharmacological actions attributed to DIP were in reality caused by the solvent.

The results of the present study clearly demonstrate that DIP potentiates inhibitory transmission in the olfactory cortex by intensifying the post-synaptic actions of GABA, the only inhibitory transmitter of this brain region that has been identified with any certainty (Pickles & Simmonds, 1976; 1978; Scholfield, 1978; Collins, 1979). First, in a proportion of slices, DIP caused a concentration-dependent increase in the area of the surface field potentials known as the late N- and I-waves (Figure 2). These potentials are the surface correlates of GABA released from populations of interneurones acting on the LOT terminals and pyramidal cells, respectively (Pickles & Simmonds, 1976; 1978; see Figure 1a). Second, in a series of experiments in which the LOT of slices was stimulated twice in rapid succession, DIP potentiated both pre- and post-synaptic inhibition evoked by the first conditioning pulse (Figure 3). Third, the depressant effect of exogenous GABA on the excitability of the LOT terminals was potentiated by DIP in a picrotoxin-sensitive manner (Figure 4). Finally, DIP was shown to potentiate the depolarizing responses evoked by GABA applied to one surface of olfactory cortex slices (Figure 5). The mechanism by which DIP potentiates GABA-mediated transmission is unlikely to involve inhibition of GABA re-uptake for the actions of muscimol and 3-aminopropanesulphonic acid, two structural analogues of GABA that are poor substrates for the GABA uptake systems (Johnston *et al.*, 1978; Brown *et al.*, 1980), were also potentiated by DIP (Figure 5).

The ability of DIP to potentiate GABA-mediated synaptic inhibition is shared by many other general anaesthetics (Kean & Biziere, 1987). When tested in the olfactory cortex, various barbiturates, including pentobarbitone (Pickles & Simmonds, 1978; Scholfield, 1978), together with chloralose, alphaxalone, ketamine, halothane and urethane (Scholfield, 1980) all potentiate inhibition with differing potencies. Drugs which potentiate GABA-mediated transmission do so by interacting with either the site at which benzodiazepines bind or with a site associated with the chloride ion channel of the $GABA_A$-receptor complex. At somewhat higher concentrations, barbiturates (Schulz & Macdonald, 1981) and alphaxalone (Barker *et al.*, 1987) directly activate a bicuculline-sensitive chloride conductance. If the potentiation of the action of GABA by DIP depends on a direct interaction with the $GABA_A$-receptor

complex, the failure of the benzodiazepine receptor antagonist Ro 15-1788 (Polc *et al.*, 1981) to block its effects (Figure 6) shows that the anaesthetic does not act at the benzodiazepine receptor site (see also Hales & Lambert, 1988). Similarly, at least at concentrations up to 200 μM, DIP does not mimic GABA when applied to slices of olfactory cortex. These findings suggest that the likely site of action of DIP is at the chloride ion channel, the site at which barbiturates and alphaxalone exert their primary effects (Barker *et al.*, 1987).

Although an intensification of GABA-mediated transmission probably contributes toward anaesthesia, it is unlikely to be the sole cause for the benzodiazepines, which are not usually considered to be general anaesthetics, potently potentiate GABA-mediated inhibition in the central nervous system. At the range of concentrations tested (up to 50 μM), DIP had no significant effect on the amplitude or latency of the monosynaptically-evoked massed excitatory postsynaptic potential, the N-wave (Figures 1a and 2). In contrast, the potentials known as the N'b'- and P-waves, which on stimulation of the LOT are evoked on di-/polysynaptic excitation of the pyramidal cells by way of the superficial and deep association fibres, respectively (Figure 1b), are markedly reduced or abolished by DIP (Figures 7 and 8). The functional significance of this was tested in a series of conditioning experiments. Submaximal stimulation of the deep association fibres evoked a P-wave which was accompanied by a reduction in the latency to peak of an N'a'-wave evoked on LOT stimulation (Figure 9). This evidence of an increase in excitability of the pyramidal cells, together with the associated P-wave, was abolished by DIP.

Experimental evidence suggests that excitatory transmission in the olfactory cortex is probably mediated by L-aspartate and/or L-glutamate (Collins, 1986) and that di-/polysynaptic excitations are mediated by excitatory amino acid receptors of the N-methyl-D-aspartate category (Collins & Howlett, 1988). The inability of DIP to antagonize the postsynaptic actions of either N-methyl-D-aspartate, or of other selective and non-selective excitatory amino acid receptor agonists (see Results), suggests that the alteration in excitatory transmission by DIP may be caused by a picrotoxin-insensitive reduction in transmitter release from the terminals of the association fibres. However, some care is necessary in the interpretation of these results as the presence of picrotoxin in these experiments might have masked a possible action of DIP on the chloride ion channel (see above) which, indirectly, might have reduced the responses evoked by the excitatory amino acids. In addition, it is difficult to understand how DIP could inhibit excitatory transmitter release from the association fibres but not the LOT, although it should be noted that presynaptic $GABA_B$-receptors are located at the former but not the latter sites (Collins & Howlett, 1988).

There is abundant evidence that general anaesthetics depress excitatory synaptic transmission in the CNS. In the olfactory cortex, several anaesthetics, including pentobarbitone, alphaxalone, diethyl ether and halothane, reduce monosynaptic transmission at the LOT-pyramidal cell synapse and most also decrease the sensitivity of neurones to glutamate (Richards *et al.*, 1975; Richards & Smaje, 1976). However, possible effects of the anaesthetics on polysynaptic excitations in the olfactory cortex have not been investigated and so it is not known whether the inhibitory effects of DIP described in this paper are characteristic of this class of compounds. In cat spinal cord, DIP inhibits polysynaptic reflexes which, it should be noted, are mediated by N-methyl-D-aspartate receptors (Lodge & Anis, 1984). The action of DIP on excitatory transmission in the olfactory cortex probably explains the variability of its effects on the late N- and I-wave field potentials (see Results), in that activation of at least some of the inhibitory GABA interneurones is a di-/polysynaptic event (see Figure 1a) also mediated by N-methyl-D-aspartate receptors (Collins, 1982).

The steady state blood level of DIP during an intravenous infusion to maintain anaesthesia in human patients has been calculated to be approximately 35 μM (Gepts *et al.*, 1985). Although the dose-dependency of the potentiation of GABA-mediated inhibition was not investigated, concentrations of DIP of 12.5 μM or less potentiated the actions of 3-aminopropanesulphonic acid (Figure 6a) and a similar range of concentrations effectively antagonized polysynaptic excitations (Figures 7 and 8). Thus during anaesthesia, a sufficient concentration of DIP will be achieved to exert profound effects on synaptic transmission. Nevertheless, it remains to be established whether potentiation of GABA-mediated inhibition and reduction of polysynaptic excitations are in any way related to the mechanism by which DIP induces and maintains anaesthesia.

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