hydrin produced are insufficient to form **VIII** (R=H), since this conjugate is not detectable after administration of the low-dose regime of α-chlorohydrin which causes infertility.

Attempts to detect the aldehyde (**II**) as a urinary metabolite have been unsuccessful though in rat liver homogenates, the conversion of α-chlorohydrin to BCLA (**III** → **IV**) has been demonstrated to involve NAD^+-linked dehydrogenase activity with the formation of an as yet unidentified aldehyde. Rats given ^{36}Cl-α-chlorohydrin after pretreatment with disulfuram to inhibit aldehyde dehydrogenase[20,21] show a delay in the onset of excretion of BCLA (**IV**) in the urine (from 5 h to 9 h) and trace amounts of an α-halohydrin aldehyde can be detected from 5 to 9 h after administration.

It is tempting to postulate differing enzyme specificities for the (+)- and (−)-isomers of the aldehyde (**II**) by the dehydrogenases **II** → **IV** and **II** → **III**. This would explain preferential BCLA and oxalic acid formation by (+)-ACP, leading to renal toxicity, and α-chlorohydrin production by (−)-ACP yielding an antifertility response since oxalic acid is detectable[22] only as a urinary metabolite of (+)-ACP and not from (−)-ACP. The comparative metabolism of (+)- and (−)-ACP in vivo and in vitro is at present under investigation.

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Comparison of bicyclic phosphorous esters with bicuculline and picrotoxin as antagonists of presynaptic inhibition in the rat cuneate nucleus

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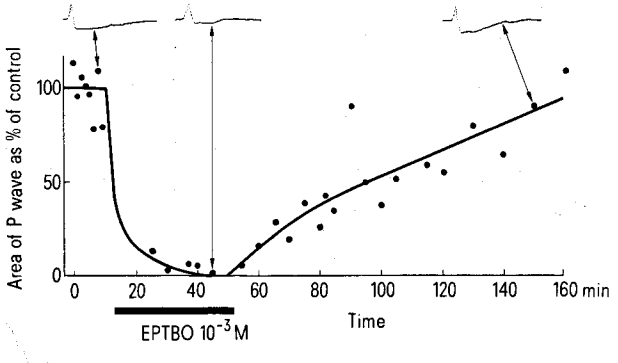
Summary. The effects of 2 of a series of bicyclic phosphorous esters, the ethyl (EPTBO) and isopropyl (IPTBO) compounds, were compared with those of the GABA antagonists, picrotoxin and bicuculline, on presynaptic inhibition in the rat cuneate nucleus. Both EPTBO and IPTBO were found to be effective, reversible antagonists of presynaptic inhibition, with IPTBO approximately 10 times more potent than EPTBO and equipotent with bicuculline, EPTBO equipotent with picrotoxin.

The γ-aminobutyric acid (GABA) antagonists, bicuculline and picrotoxin, have been reported to be effective antagonists of presynaptic inhibition in the vertebrate spinal cord and dorsal column nuclei[2–6]. In addition both picrotoxin and bicuculline block the direct depolarizing actions of GABA on primary afferent terminals[6–8] suggesting that the blocking of presynaptic inhibition by these substances is due to a discrete action at GABA-nergic receptors on primary afferent terminals. It is interesting therefore to examine the effect on presynaptic inhibition of a new group of substances, the bicyclic phosphorous esters, which have recently been reported to be effective GABA antagonists in the rat superior cervical ganglion[9].



Depression and recovery of the cuneate P wave during application and after removal of 10^{-3} M EPTBO. The sample recordings of the computer-averaged cuneate evoked potential shown above the graph are taken from left to right, before application of EPTBO, during maximum depression of the P wave in the presence of EPTBO and almost 2 h after its removal, as indicated. Each computer record is an average of 16 successive recordings of the supramaximal response to ipsilateral forepaw stimulation; sweep time is 100 msec. The first negative, upward going component of the response (the N wave) is unaffected by EPTBO, while the second, positive going component (the P wave) is virtually completely abolished. The graph below the computer records shows the time course of depression and recovery expressed in terms of the area of P wave as a percentage of its mean control size.

Materials and methods. Adult rats were anaesthetised with a 1% chloralose–10% urethane solution (0.8 ml/100 g i.p.) and the pial surface of the dorsal column nuclei exposed for electrical recording and drug application as described elsewhere[6]. Evoked potentials were recorded from the pial surface of the cuneate nucleus following supramaximal electrical stimulation of the ipsilateral forepaw with a 0.1 msec stimulus delivered through p.c. needle electrodes. Groups of 16 successive evoked potentials were computer-averaged during superfusion of the pial surface with an artificial cerebrospinal fluid (csf) solution[10] or with similar solutions containing 10^{-3} or

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10^{-4} M picrotoxin, bicuculline or one of the bicylic phosphorous esters 4-ethyl-1 phospha 2,6,7-trioxabicyclo (2,2,2) octane (EPTBO) or its isopropyl equivalent (IPTBO).

Evoked presynaptic inhibition was estimated quantitatively as the area enclosed by the large positive-going component (P wave) of the cuneate evoked potential, which is known to reflect the underlying depolarization of primary afferent terminals, the basic mechanism of vertebrate presynaptic inhibition. Alterations in the size of the P wave have frequently been used in pharmacological investigations of presynaptic inhibition[11] but computer-averaging of the evoked response adds greater reliability to the technique.

Results and discussion. Both EPTBO and IPTBO were effective antagonists of presynaptic inhibition, reducing or abolishing the cuneate P wave. In both cases the onset of action was apparent within a few min of the superfusion fluid being applied to the cuneate and the maximum effect was reached within 30–40 min for 10^{-3} M EPTBO (figure) and 10^{-4} IPTBO, the latter being consistently about 10 times more potent than EPTBO. After removal of EPTBO or IPTBO from the superfusion fluid, the depressant effects were fully reversible within a period of about 2 h. There was no consistent effect on the preceding N wave component of the evoked response (figure). The depressant action of picrotoxin on the P wave was approximately equipotent with that of EPTBO, bicuculline with IPTBO. In contrast to the bicyclic phosphorous esters, however, the actions of picrotoxin and bicuculline were either only partially reversible or irreversible over the time course of the experiments, as has been reported elsewhere[5,6].

The bicyclic phosphorous esters are potent convulsants, block the depressant actions of GABA on single neurones in the rat medulla and the depolarizing action of GABA on superior cervical ganglion cells with the relative potencies of IPTBO = 1, EPTBO = 0.1[9]. The data reported here extend these observations to include antagonism of presynaptic inhibition with the relative potencies remaining unchanged. It is likely that the convulsant activity of the bicyclic phosphorous esters depends to a considerable extent on their ability to antagonize presynaptic inhibition. The observations reported here also support the theory that GABA is the presynaptic inhibitory transmitter[6].

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The effect of ion pair formation on the antimuscarinic activity of methantheline

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Summary. The quaternary ammonium compound, methantheline, was found to antagonize acetylcholine induced contractions in isolated guinea pig ileum by a mechanism which did not conform to the criteria for either competitive or non-competitive inhibition. Enhancement of the lipid solubility of methantheline by formation of an ion pair with trichloracetate failed to influence its cholinergic inhibitory activity. The results suggest that in the guinea pig ileum a) an intracellular site of action does not exist for methantheline and b) the membrane receptors for methantheline most likely are located in an aqueous environment.

Ion pairing is a technique which has been used to mask the charged site on an ionic species and thereby render it more lipid soluble[3–5]. The potential application of this technique as a means of improving the gastrointestinal absorption of highly ionized drugs has been demonstrated by Irwin et al.[6] who observed an increase in rate of onset and in intensity of mydriatic response following oral administration of the quaternary ammonium compound isopropamide iodide, in combination with the ion pair forming counter ion trichloracetate. A change in the physical properties of a charged drug molecule conceivably could influence its pharmacologic activity in other ways such as by increasing its accessibility to intracellular sites of action or by altering its affinity for membrane receptors. The present investigation was undertaken to evaluate the effects of ion-pair formation on the lipid solubility and antimuscarinic activity of methantheline bromide, a quaternary ammonium compound which is highly ionized at physiological pH.

Materials and methods. The effect of ion pair formation on lipid solubility was examined by 2 methods. As a direct measurement, the partition coefficient for methantheline was determined in the presence of excessive molar concentrations of trichloroacetate (TCA). An indirect assessment of the ability of complex formation with TCA to enhance the lipid solubility of methanteline was made using an in situ rat intestinal loop preparation[7]. 4.76 mM ^{14}C-methantheline (specific activity, 1.25×10^7 cpm/mg) in the presence of excessive molar concentrations of TCA was introduced into the intestinal lumen and the amount remaining was determined at different times over a 1-h-period using a Packard Tricarb liquid scintillation counter.

The effect of ion pair formation on the antimuscarinic activity of methantheline was investigated by the following procedure. Female guinea-pigs weighing 200–300 g were sacrificed by cervical dislocation and the abdominal cavity opened by midline laparotomy. A 2–3 cm segment of distal ileum was excised and trimmed of attached mesentery. The sample was suspended in a 15 ml isolated tissue bath containing Tyrode's solution of the following millimolar composition: NaCl, 137; KCl, 2.7; $CaCl_2$, 2.5; $NaHPO_4$, 4.2; $MgSO_4$, $7H_2O$, 8.3; glucose, 10; $NaHCO_3$, 14. The bath was maintained at 37 ± 1 °C and continuously bubbled with 95% O_2–5% CO_2. The system was arranged in such a way that washout of drug solutions could be accomplished without removing tissue samples from the bath. Contractions induced by varying doses of acetylcholine chloride were monitored using a Grass FT03C force-tension transducer in conjunction with a Hewlett-Packard 7404A oscillographic recorder.

An ion pair solution was prepared by combining meth-