The metabolism of the male antifertility agent 1-amino-3-chloropropan-2-ol in the rat

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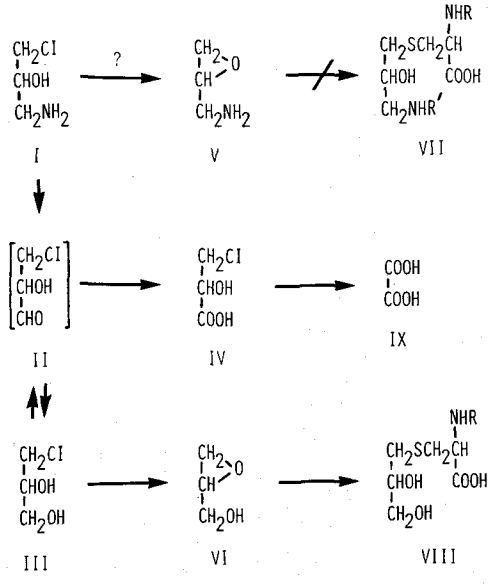
Summary. The male antifertility agent 1-amino-3-chloropropan-2-ol (ACP, **I**) has been shown to be metabolized to α-chlorohydrin (**III**) and metabolites of α-chlorohydrin. This accounts for the similar antifertility and renal toxicity effects of both compounds.

In many species, low doses of α-chlorohydrin (3-chloropropane-1,2-diol, **III**) produce an immediate and reversible phase of infertility in the male by an action on epididymal sperm[1], presumably by glycolytic inhibition[2]. It has been suggested[3] that this low-dose action involves the epoxide metabolite glycidol (2,3-epoxypropanol, **VI**) and that exhibition of renal toxicity with higher doses of α-chlorohydrin is due to an oxidative metabolic pathway[4] to β-chlorolactic acid (BCLA, **IV**) and, ultimately oxalic acid (**IX**). Although many structure-activity studies[5–7] have been reported with compounds related to the α-chlorohydrin structure, only one analogue (±)-1-amino-3-chloro-propanol, (ACP, **I**) has shown any comparable antifertility effect[8,9]. The resolution of (±)-ACP into its isomers has shown the (+)-isomer to be toxic and inactive as an antifertility agent and the (−)-isomer to be devoid of toxicity but to possess antifertility activity. On a weight basis, (−)-ACP is not as active as α-chlorohydrin[10] although both compounds elicit similar effects and do not cause genetic damage[11]. As these results suggest that the metabolism of ACP could be linked to that of α-chlorohydrin, the metabolism of (±)-ACP was investigated to determine whether common intermediates were responsible for the antifertility action and the renal toxicity of both compounds.

Chromatograms of urine collected over 250 h from i.p. administration of ^{36}Cl-(±)-ACP[12] (100 mg/kg) to male Wistar rats, showed 4 radioactive areas identified[14] as Cl^- (appearing in urine from 40 to 250 h from administration), BCLA (48–72 h) unchanged ACP (0–72 h) and α-chlorohydrin (0–6 h). This indicates that ACP is degraded to β-chlorolactaldehyde (**II**) which, by oxidation, is converted to BCLA (**IV**) and, by reduction, to α-chlorohydrin (**III**). Approximately 66% of the radioactivity is excreted over 72 h. Of this, 94% is unchanged (±)-ACP, 2.5% is α-chlorohydrin and 3% BCLA. The oxidation of (±)-ACP to the aldehyde (**II**) is thought to involve monoamine oxidase as pretreatment of animals with the MAO-inhibitor mebanazine[17] had no effect on the excretion of unchanged ACP but delayed the urinary appearance of α-chlorohydrin and BCLA to 24–41 h and 65–72 h respectively.

Extraction of urine collected over 5 days from repeated daily oral administration of (±)-ACP (100 mg/kg), subsequent processing and amino-acid analyses[18], did not reveal any cysteine conjugates corresponding to **VII** (R=$COCH_3$, R′=H or R=R′=$COCH_3$) or **VIII** (R=$COCH_3$). This indicates that ACP does not alkylate cysteine (glutathione)[19] and that the amounts of α-chloro-



The metabolism of ACP and α-chlorohydrin.

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12 Hydrolysis of 1-(N-phthalimido)-2,3-epoxypropane[13] in $H^{36}Cl$ gave ^{36}Cl-ACP as the $H^{36}Cl$ salt. Data presented here have been corrected for the excretion of $H^{36}Cl$.
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14 Chloride ion was identified by the method of Seiler and Kaffenberger[15], BCLA and α-chlorohydrin according to Jones[16] and ACP by means of a Jeol amino acid analyzer.
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18 Urine was chromatographed on Whatmans 3MM paper in n-butanol:acetic acid:water (4:2:1) and areas corresponding to authentic **VII** (R=$COCH_3$ R=H, R=R′=$COCH_3$ and R=R′=H) and **VIII** (R=H and R=$COCH_3$) eluted with water. Where acetylated derivatives were suspected, acid hydrolysis (5NHCl, 90 °C, 1 h) was performed. All extracts were purified on Dowex-50W columns and examined on a Jeol amino acid analyzer against authentic **VII** (R=R′=H) and **VIII** (R=H). The sensitivity of this procedure was such that it was capable of detecting less than a 0.5% conversion of **I** to **VII** and **VIII**.
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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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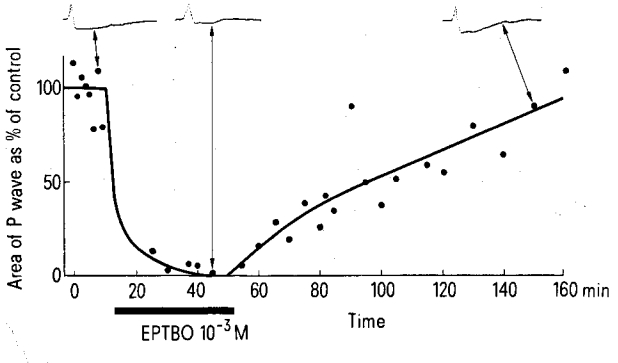
Summary. The effects of 2 of a series of bicyclic phosphorous esters, the ethyl (EPTBO) and isopropyl (IPTBO) compounds, were compared with those of the GABA antagonists, picrotoxin and bicuculline, on presynaptic inhibition in the rat cuneate nucleus. Both EPTBO and IPTBO were found to be effective, 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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