The free chrysanthemic acids and allethrolones, and the pyrethroids permethrin and S-5602[16] were negligible competitors at their solubility limits. Even when 1R, 3R chrysanthemic acid was esterified to 5-benzyl-3-furyl methanol or 3-phenoxybenzyl alcohol[16] (bioresmethrin and (+)-trans phenothrin, respectively), little competition was observed. However, an extract of *Chrysanthemum* flowers containing ~43% pyrethrins[17] (all of which closely resemble S-bioallethrin) gave 50% radioactivity displacement at 1.5 nmoles/assay. These experiments indicate that this antibody population selectively recognized the entire S-bioallethrin molecule.

Antibody stereoselectivity for the haptenic optical or geometric configuration thus represents a powerful potential tool to the environmental chemist. Development of RIA's for pesticides may not only simplify and improve detection of parent compound residues, but may also be applied to metabolism studies, monitoring of compound shelf life, and forensic toxicology.

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Effect of hydroxydopamine on the morphine-induced reduction in brain acetylcholine turnover

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Summary. Morphine reduced brain acetylcholine turnover in normal and 6-hydroxydopamine-pretreated rats and mice. Morphine probably has a direct effect on cholinergic neurons rather than modifying acetylcholine indirectly through catecholamine neurons. Acetylcholine is not directly involved in morphine's antinociceptive action in the mouse but it could be implicated in the rat.

Morphine interacts with brain acetylcholine (Ach). It reduces Ach release in vivo[1] and in vitro[2], increases brain Ach content[3] and reduces its turnover[4,5]. It is not clear whether cholinergic neurons are affected directly by morphine or if Ach has a major role in morphine's antinociceptive action. Interactions occur in brain between cholinergic and catecholaminergic neurons[6] so it is possible that morphine could have an indirect effect on Ach. There is an established connection between morphine, Ach and the catecholamines because dopamine drugs alter Ach turnover[7] and morphine increases the turnover of brain dopamine[8]. Drugs that affect brain catecholamines alter morphine's antinociceptive action[9]. 6-Hydroxydopamine (6-OHDA), which depletes brain noradrenaline and dopamine[10–12], antagonises morphine's antinociceptive action in mice[13]. The possibility that Ach could have a direct, major role in morphine's antinociceptive action has been investigated by measuring morphine-induced reductions in Ach turnover in rats and mice pretreated with 6-OHDA.

Materials and methods. Female Sprague-Dawley rats (150–155 g) and male albino mice (25–35 g) were used. Rats, anaesthetized with ether, received 2 intraventricular injections[14] of either saline solution or 6-OHDA (250 µg) 48 h apart. Mice, anaesthetized with ether, received 2 doses of 6-OHDA (75 µg) intraventricularly[15]. 10 days were allowed after the 6-OHDA for the depletion of catecholamines. The turnover of Ach was estimated by measuring Ach levels following inhibition of its synthesis with hemicholinium-3 (HC-3)[4]. Groups of saline- and 6-OHDA-pretreated rats and mice were given 16 mg kg^{-1} of morphine i.p. 10 min before HC-3 intraventricularly[14,15]. Each rat received 20 µg of HC-3 and the mice received 1 µg for each 15 g of b.wt. The animals were killed 30 min later using the brain rapid ejection and freezing apparatus[16]. The discs of frozen brain were extracted in 5 ml g^{-1} of acidified ethanol solution[17] and the Ach content was measured by bioassay. Rats and mice pretreated with saline or 6-OHDA intraventricularly were killed by decapitation for the fluorimetric measurement[18] of brain noradrenaline and dopamine.

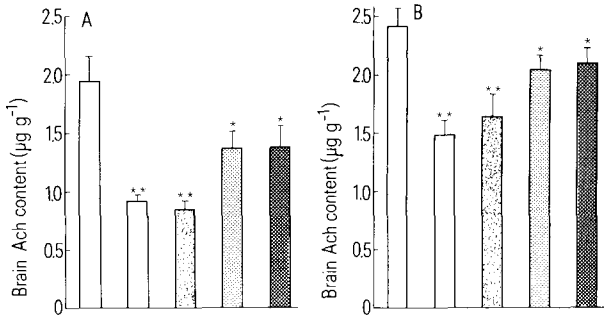
Brain noradrenaline and dopamine in rats and mice following intraventricular administration of saline and 6-OHDA

Species	Treatment	Noradrenaline (µg g^{-1} ± SEM)	Dopamine (µg g^{-1} ± SEM)
Rat	Saline	0.32 ± 0.02 (7)	1.09 ± 0.03 (6)
Rat	6-OHDA	0.07 ± 0.01[b] (8)	0.75 ± 0.03[a] (6)
Mouse	Saline	0.44 ± 0.02 (6)	1.31 ± 0.04 (6)
Mouse	6-OHDA	0.16 ± 0.02[b] (6)	0.70 ± 0.03[a] (6)

Significance of difference between saline- and 6-OHDA-treated. (Student's t-test) [a] $p < 0.01$; [b] $p < 0.001$. Number of animals used shown in brackets.

Results and discussion. The principle of measuring Ach turnover by inhibiting its synthesis with HC-3 has been established[4,19] and is based on the fact that HC-3 reduces brain Ach content[17]. Drugs that reduce Ach turnover antagonise the HC-3-induced depletion. Because rapid post-mortem changes in Ach levels can occur the brains were removed from the animals and frozen in less than 1 sec[16]. As can be seen from the figure, HC-3 caused identical reductions in brain Ach in rats pretreated with saline or 6-OHDA. This Ach depletion was partly prevented by morphine which is consistent with an earlier finding[4] that morphine reduces Ach turnover in rat brain. Morphine reduced Ach turnover by exactly the same amount in 6-OHDA-treated rats, which have degenerated catecholamine neurons[10-12], as in saline-treated animals. This finding implies that dopamine is not involved in the action of morphine on Ach turnover. The results are consistent with a direct action of morphine on Ach neurons. An analysis of brain catecholamines confirmed, as shown in the table, that 6-OHDA caused a substantial depletion of noradrenaline together with a reduction in dopamine. Because 6-OHDA neither effects morphine's antinociceptive action in rats[13] nor prevents the action of morphine on brain Ach it is possible that the antinociceptive action of morphine in the rat is partly dependent on cholinergic neurons. Morphine neither reduces Ach release nor induces analgesia in rats with raphe lesions[20].

Morphine reduced Ach turnover to the same extent in normal and 6-OHDA-treated mice. The depletion of catecholamines by 6-OHDA in the mouse was similar to the rat so morphine probably has a direct action on cholinergic neurons. Because 6-OHDA antagonises morphine's antinociceptive action in mice[13] but does not prevent morphine from reducing Ach turnover it is unlikely that Ach has a major, direct role in mediating morphine's antinociceptive action in this species. A reduction in brain Ach with HC-3 only slightly reduces morphine analgesia in mice[21].



Effect of morphine on brain acetylcholine (Ach) turnover in rats (A) and mice (B). Normal and 6-OHDA-pretreated animals received either saline, HC-3 or HC-3 and morphine. Open columns = Ach content of normal animals; closed columns = HC-3 treated; upper left cross-hatched columns = 6-OHDA + HC-3; dotted columns = morphine + HC-3; upper right cross-hatched columns = morphine + 6-OHDA + HC-3. Ach turnover is reflected by the amount of Ach depletion produced by HC-3. Each result is the mean ± SEM obtained with 6 animals. Significance of difference between normal and treated groups: * $p < 0.01$; ** $p < 0.005$.

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Hepatic silver binding protein (Ag BP) from sparrow *(Passer domesticus)*

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Summary. A silver binding protein (Ag BP) has been identified in the liver of sparrows administered a tracer dose of ^{110m}Ag. The protein as purified by gel-filtration shows a major absorption maximum at 260 nm and a minor one at 225 nm. It has a mol.wt of 9500 daltons and is stable when exposed to high temperature (64 °C for 15 min) as well as to acidic pH (2.2).

A low molecular weight protein having high cysteine (upto 30%) and metal (upto 11%) content has been identified in the kidney and liver of a number of mammalian[1-5] as well as other species[6,7]. Proteins named metallothioneins have been shown to bind a number of heavy metals, viz Ag^+, Zn^{++}, Cd^{++} and Hg^{++}. The present interest in metallothioneins arises out of their suggested roles in heavy metal detoxification[8] and normal zinc homeostasis in mammals[9]. Here, we report the identification of a low mol.wt silver binding protein which shows heat stability and low absorption at 280 nm. The silver complex of the protein is also stable at pH 2.2.

Materials and methods. ^{110m}Ag as $AgNo_3$ (sp. act. 168 mCi/g Ag) was obtained from Bhabha Atomic Research Centre,