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Centrally-mediated hyperalgesia in mice produced by the antimuscarinic agents atropine and hyoscine

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It is well known that centrally active cholinomimetic agents can induce antinociceptive activity comparable to morphine in rodents (George et al 1962; Harris et al 1968). This activity is believed to be of a central origin (Metys et al 1969) and is effectively antagonized by centrally active antimuscarinic agents (Ireson 1970) though the precise mechanism is not totally understood.

Recently, we developed a reduced temperature tail immersion nociceptive method through which we were able to screen drugs qualitatively and quantitatively for their ability to produce hyperalgesia (Gonzalez et al 1980). The purpose of the present study, therefore, was to determine whether the antimuscarinic agents atropine and hyoscine possessed any inherent centrally mediated hyperalgesic activity in the test, in order to gain some insight into the mechanism of antinociception produced by cholinomimetics.

Male albino mice, GB1 variants of an ICI derived strain, 18–20 g, were allowed free access to a standard 41B cube diet and water, both being withdrawn 2 h before the first experimental results were recorded.

Nociceptive sensitivity was determined using a modified version of the tail immersion technique of Sewell & Spencer (1976). Briefly, the procedure involved the immersion of the whole tail of the animal in a water bath maintained at 45 °C, the response latency being determined in seconds when the stimulus was applied to each animal at 20 min intervals for 120 min. A cut-off time of 20 s was imposed during the measurement of response latencies to avoid any possibility of permanent tissue damage.

From the time course of the effect of each dose of drug, an index termed '% Hyperalgesia' was calculated using the following formula:

$$\% \text{ Hyperalgesia} = \frac{D-C}{D} \times 100$$

where D and C represents the area under the curve (integral of the effect (reaction time) (s) vs experimental duration (min) for drug treated and 0·9% NaCl(saline)-treated animals respectively). In all experiments statistical tests of significance for differences between various groups were carried out using Student's *t*-test and significance was assumed at the $P < 0.01$ level.

Animals injected subcutaneously (s.c.) with varying doses of atropine sulphate or hyoscine hydrobromide displayed consistent and significantly more rapid nociceptive reaction times than concurrently tested saline-treated controls (n = 30). The peak hyperalgesic effect occurred between 20 and 40 min and was not detectable 3 h after treatment. Calculation of % hyperalgesic effects over 120 min revealed that graded responses were produced at doses up to 3·0 mg kg^{-1} of atropine sulphate and 5·0 mg kg^{-1} of hyoscine hydrobromide. Conversely, atropine methylnitrate had no significant hyperalgesic effects up to 10·0 mg kg^{-1} s.c. at which dose there is pronounced blockade of peripheral cholinergic activity (Albanus 1970) (see Table 1). When atropine methylnitrate was injected intracerebroventricularly (i.c.v.) at 5 and 10 μg kg^{-1}, a significant reduction ($P < 0.01$) was noted in the nociceptive reaction times compared with saline-treated controls (Fig. 1). This effect was dose-related and lasted for over 120 min thus contrasting with the effects produced by peripherally administered doses of atropine methylnitrate.

In the present study employing a wet heat noxious stimulus, significant dose-related hyperalgesic activity following peripheral doses of atropine and hyoscine was readily demonstrable. However, the quaternary muscarinic antagonist atropine methyl nitrate, an agent which has reduced tendency to cross the blood-brain barrier, failed to significantly alter nociceptive sensitivity when administered peripherally. Conversely, when this agent was injected intracerebroventricularly a marked dose-related hyperalgesia was produced thus suggesting that the site of atropine's action was central in origin.

There are three possible explanations for the decreases in nociceptive reaction times observed after atropine and hyoscine. Firstly, these agents may stimulate the

Table 1. Hyperalgesic effect of peripherally administered antimuscarinic agents in the mouse tail immersion test (45 °C).

Treatment Drug	Dose (mg kg^{-1})	% Hyperalgesic effect ± s.e.m. (n)	P*
Atropine sulphate	0·3	35·3 ± 3·0 (10)	<0·001
	1·0	45·7 ± 4·3 (8)	<0·001
	3·0	66·9 ± 3·3 (10)	<0·001
Hyoscine hydrobromide	0·5	28·5 ± 4·0 (10)	<0·001
	1·0	35·1 ± 6·6 (9)	<0·001
	5·0	48·9 ± 2·4 (10)	<0·01
Atropine methylnitrate	2·5	7·5 ± 3·4 (8)	NS
	5·0	10·5 ± 6·0 (10)	NS
	10·0	8·0 ± 2·2 (10)	NS

* Compared with controls.
NS–non significant.

* Correspondence.

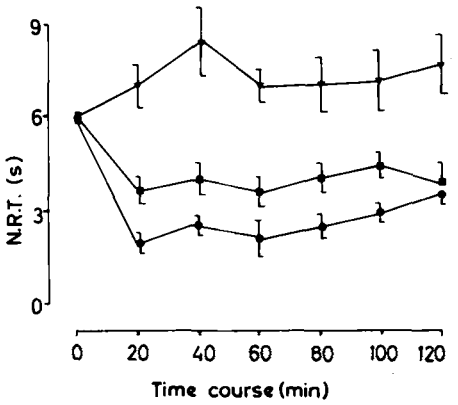

FIG. 1. The effect of centrally administered saline (▼——) and atropine methylnitrate (■—— 5 $\mu g\ kg^{-1}$, ●—— 10 $\mu g\ kg^{-1}$) on nociceptive reaction times (N.R.T.) in mice (n = 10).

release and central turnover of acetylcholine via muscarinic antagonism and this would consequently lower brain acetylcholine concentrations (Sparf 1973). Alternatively, these antimuscarinics may somehow interfere with the release of endogenous opioids which would normally be pain suppressive (Akil et al 1978). Although endorphins do not universally modify cholinergic activity in the c.n.s. there are specific cholinergic pathways which are believed to be under enkephalinergic control (Wood & Stotland 1980). Thirdly, since Zakusov (1980) suggested that tropane derivatives may be opiate receptor ligands and thus reduce or eliminate the effects of endogenous opioids, it is conceivable that atropine and hyoscine may produce hyperalgesia partially by a direct opiate receptor mechanism.

The inhibitory effect of atropine on the analgesia produced by the cholinomimetic oxotremorine has been previously demonstrated (Ireson 1970; Ben-Sreti & Sewell, unpublished observations). This antagonism was proposed by Campbell et al (1970) to be partly due to simple competitive antagonism at muscarinic receptors. Additional supportive evidence for this was derived from the fact that larger doses of oxotremorine surmounted the inhibitory effects of atropine. Furthermore, an earlier report by Crossland & Slater (1968) hypothesized that atropine reversal of oxotremorine analgesia was the result of a change in the status of 'free' and 'bound' acetylcholine induced by the muscarinic antagonist.

In the light of the present findings regarding antimuscarinic hyperalgesia we are prompted to hypothesize that, in addition to the previously discussed mechanisms, atropine may in part antagonize oxotremorine analgesia through its inherent hyperalgesic properties and this is considered a functional antagonism. Moreover it might be suggested that there may be a tonic cholinergic mechanism related to nociceptive thresholds in mice although this requires further investigation.

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