The effect of 6-hydroxydopamine on the antinociceptive action of analgesics in mice

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It has been established that the administration of compounds such as α-methyltyrosine which alter brain catecholamine content may also change the animals sensitivity to the antinociceptive action of morphine (Verri, Graeff & Corrado, 1968; Major & Pleuvry, 1971; Buxbaum, Yarbrough & Carter, 1973). It is now customary to administer 6-hydroxydopamine (6-OHDA) to produce a depletion of cerebral catecholamines since this has the advantage of not affecting other known transmitter substances in the brain (Jacks, Champlain & Cordeau, 1972). When 6-OHDA is injected into the cerebral ventricles of rats and mice it causes the catecholamine containing neurons to degenerate with the result that there is a prolonged, possibly permanent, depletion of noradrenaline, and to a lesser extent dopamine, from the brain (Bloom, Algeri & others, 1969; Breese & Traylor, 1970, 1971; Uretsky & Iversen, 1970).

Changes in the sensitivity of mice to morphine's antinociceptive activity have been demonstrated following pretreatment with 6-OHDA (Ayhan, 1972; Samanin & Bernasconi, 1972; Slater, 1974). We have now compared the responses of normal and 6-OHDA-pretreated mice to several other analgesics to determine whether the reduced sensitivity to morphine produced by 6-OHDA is applicable to all analgesics or whether it is unique to morphine.

Female albino mice (Manchester University strain), 28–32 g, lightly anaesthetized with ether, were injected in the left lateral cerebral ventricle with two doses of 75 μg of 6-OHDA (as the hydrobromide) 48 h apart. The injections were made at the coordinates described by Haley & McCormick (1957). Control mice received instead 10 μl of the vehicle: ascorbic acid (1 mg ml^{-1}) in sterile, isotonic saline solution. The analgesics were administered 21 days after the first injection of 6-OHDA. Antinociceptive activity was measured by recording the time between placing the animal on the hot plate (50 or 55°) and the response: licking or raising the paws or attempting to jump off the hot plate. A cut-off time of 30 s was used except when the plate temperature was lowered to 50° when 60 s maximum exposure was allowed. The analgesics administered intraperitoneally were morphine sulphate, pethidine hydrochloride, pentazocine, levorphanol tartrate and methadone hydrochloride. The doses of the drugs are expressed as the base. Hot plate reaction times were measured immediately before and 30 min after drug administration. ED50 doses, defined as the dose of each drug required to produce 50% inhibition of the nociceptive response were calculated as described by Ankier (1974) and statistical significance was tested using the Student's *t*-test.

Dose-response curves for the analgesic drugs used are plotted in Fig. 1. The antinociceptive activity of morphine is confirmed by the dose-dependent increase in hot plate reaction time which occurred 30 min after injection. The reduced sensitivity to morphine produced by 21 days pretreatment with 6-OHDA is demonstrated by a shift to the right of the dose-response curve and the response times recorded were significantly less in 6-OHDA-treated mice compared with controls at all doses tested. Pethidine, methadone and levorphanol also produced dose-dependent inhibition of the antinociceptive response and in every instance the antinociceptive activity of the drug was significantly attenuated in 6-OHDA-pretreated mice. The antinociceptive activity of pentazocine was also signifi-

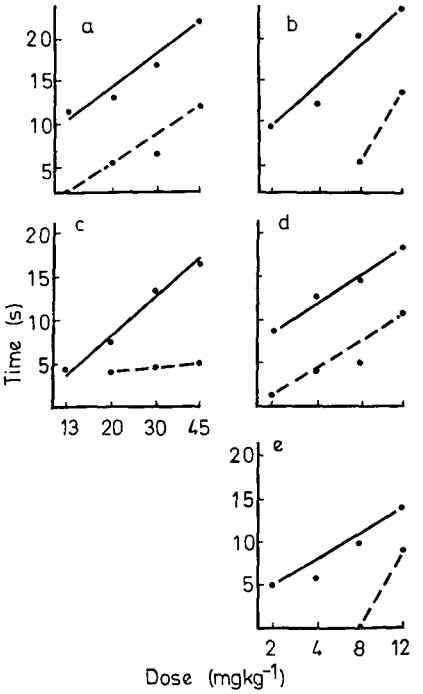

FIG. 1. Dose-response curves for several analgesics in mice. The mean increase in reaction time(s) on the hot plate was obtained using groups of 12 mice. Pretreatment was either intraventricular saline (● —— ●) or 6-OHDA (● - - - ●). a—Morphine, b—methadone, c—pethidine, d—levorphanol, e—pentazocine.

* Correspondence.

cantly antagonized by 6-OHDA treatment though, as reported by Ankier (1974), its antinociceptive activity in the hot plate test was unreliable when the plate was at the customary 55° and it was not possible to construct a dose-response curve. At 50° then pentazocine produced a dose-dependent inhibition which was significantly reduced in 6-OHDA-pretreated animals.

This was calculated using the method described by Ankier (1974). The calculated ED50 values are shown in Table 1. In every instance the result of 6-OHDA pretreatment was to cause a significant increase in the ED50 value.

Table 1. *The effect of 6-OHDA on the antinociceptive activity of analgesics in mice.*

Analgesic	Antinociceptive activity (ED50 values, 95% confidence limits, mg kg^{-1}) Normal	6-OHDA treated
Morphine	2·8 (2·2–3·6)	8·8* (6·1–12·7)
Pethidine	8·0 (7·1–9·0)	18·1* (12·1–27·2)
Methadone	4·0 (2·2–7·2)	10·2* (6·8–15·3)
Levorphanol	2·0 (1·4–2·8)	10·0* (8·6–11·6)
Pentazocine	5·0 (3·8–6·7)	15·1* (11·8–19·3)

Significance of difference between normal and 6-OHDA pretreated: $*P < 0.01$.

It is clear that the ability of 6-OHDA to antagonize the antinociceptive action of morphine is not confined to morphine alone. It has been shown previously that oxotremorine, the potent cholinomimetic which is not recognized to be an analgesic but which nevertheless strongly inhibits the hot plate nociceptive response, is also effectively antagonized by 6-OHDA (Slater, 1974). Since the actions of 6-OHDA in the central nervous system are believed to be aimed specifically at catecholamine-containing neurons, it is probably safe to assume that brain neurons releasing either noradrenaline or dopamine are necessary for the full expression of the nociceptive response.

It must not be forgotten though, that 5-hydroxytryptamine (5-HT) has periodically been implicated in the antinociceptive action of morphine (Tenen, 1968; Samanin, Gumulka & Valzelli, 1970). One reason is because an increase in brain 5-HT potentiates morphine's antinociceptive action (Rogers & Thornton, 1969; Major & Pleuvry, 1971; Sparkes & Spencer, 1971). However, a much more significant finding in relation to the results presented here is that midbrain raphé lesions, which deplete forebrain 5-HT, whilst antagonizing the antinociceptive action of morphine fail to produce any effect upon the action of other analagesics (Samanin, Ghezzi & others, 1973). The published evidence therefore shows that a normal concentration of brain 5-HT is not essential for the antinociceptive action of narcotic analgesic drugs. For this action, however, the above experiments indicate that some cerebral catecholamine containing neurons are necessary.

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