difference in the T/M ratio among the rates ranging from 8 to 16 mL min^{-1}. The present flow-dependence of pulmonary uptake and/or clearance of propranolol might not be due to any unphysiological effect of the lowest flow, since there was, for example, no evidence for either hypoxia or oedema of the tissue perfused at 4 mL min^{-1}.

From the present results, it was suggested that the in-vitro pulmonary clearance of propranolol might not be dependent on the extent of plasma protein binding but be largely dependent on the change in the lung blood flow.

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Analgesic effects of 3-methoxybenzamide in rats

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Abstract—The i.p. injection of 3-methoxybenzamide (3-MBA) in rats produces a dose-related elevation of the threshold for response to a painful stimulus. Metoclopramide, also a substituted benzamide, has analgesic activity that is attenuated by bromocriptine, a dopamine receptor agonist, and by the narcotic antagonist, naloxone, suggesting involvement of dopamine and opiate receptors in the action of this drug. The involvement of these receptors in the analgesic action of 3-MBA has been examined using L-dopa and naloxone. Neither significantly altered the analgesic action. Although the results are preliminary, the analgesic action of 3-MBA would not seem to occur via opiate or dopamine receptors.

In a study of the tumour-promoting effects of 3-methoxybenzamide (3-MBA) in hamsters, the drug appeared to make the animals insensitive to pain, inducing a state closely resembling general anaesthesia (Miller et al 1986). When the drug was administered to rats, the anesthetic-like state did not ensue, but the animals were lethargic and less sensitive to painful stimuli. Experiments were therefore designed to evaluate this apparent analgesic effect in rats. Since there is evidence for the involvement of opiate and dopaminergic receptors in the analgesic effects of metoclopramide, a substituted benzamide (Ramaswamy & Bapna 1986), the potential roles of these receptors in the analgesic effects of 3-MBA were investigated.

Materials and methods

Male outbred Sprague-Dawley rats, ca 250 g, were used. Tests for analgesia were as described by Swingle et al (1971) using an "Analgesy-Meter" (Varese, Italy), which provides a measure of pain threshold by increasing amounts of pressure applied to the web structure of the paw until the paw is withdrawn. Baseline or predrug responses to paw pressure were determined on forty rats. Subsequently, these rats were divided into five equal groups. Groups I, II, and III were injected i.p. with either 100, 200 or 300 mg kg^{-1} 3-MBA (Aldrich Chemical CO., Milwaukee, WI) prepared in 100% DMSO (Sigma Chemical Co., St. Louis, MO). The response (pain threshold) was measured as before at 0·5, 1·5 and 2·5 h postinjection. Groups IV and V were similarly treated with 100% DMSO or physiological saline, respectively, and tested for pain threshold as before. The means of the measurements of each group at each time interval were compared (using the Student's *t*-test) to the means from the DMSO controls. DMSO is known to produce mild analgesia in animals (Haigler & Spring 1983), thus, the means of the DMSO treated animals were also compared with saline treated animals.

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To test for the possibility that 3-MBA produced its effects via opiate receptors, eight male rats were given 300 mg kg^{-1} 3-MBA as before and the analgesia test applied at 0·5 h post-injection. Immediately following the 0·5 h test, each rat received 0·5 mg kg^{-1} naloxone HCl (DuPont Pharmaceuticals, Manati, Puerto Rico). The analgesia test was repeated at 1·5 and 2·5 h after 3-MBA injection.

To test for dopaminergic involvement in the 3-MBA induced effects, the above procedure was repeated replacing naloxone, with an injection (i.p.) of 50 mg kg^{-1} L-dopa (Sigma Chemical Co.) + 20 mg kg^{-1} carbidopa (kindly provided by Merck Sharp and Dohme, West Point, PA) to increase brain levels of dopamine. The drug combination was prepared in 0·1 M HCl and adjusted to a pH of 5 with 5 M NaOH. Ascorbic acid was added (45 mg mL^{-1}) as an antioxidant. The data from both the naloxone and L-dopa experiments were compared with those previously obtained with 300 mg kg^{-1} 3-MBA alone.

Results

Rats treated with 3-MBA at 300 mg kg^{-1} were lethargic, but, in contrast to the behaviour of hamsters noted earlier, none had a loss of righting reflex or exhibited a state that could be regarded as general anaesthesia. A standard catalepsy test (Morpurgo 1965) gave negative results. The effects of the three doses of 3-MBA are shown in Fig. 1. Consistent with a report by Haigler

Table 1. Effects of naloxone and L-dopa on 3-MBA-induced analgesia in rats. Data are pain thresholds in grams expressed as means ± s.e. Naloxone and L-dopa were given at 0·5 h.

Treatment	n	Time (h) 0	0·5	1·5	2·5
3-MBA, 300 mg kg^{-1}	8	151·3 ± 11·41	475·0 ± 25·0	450·0 ± 32·24	401·25 ± 42·86
3-MBA, 300 mg kg^{-1} + naloxone, 0·5 mg kg^{-1}	8	129·4 ± 16·91	453·8 ± 24·42	408·8 ± 31·02	380 ± 49·68
3-MBA, 300 mg kg^{-1} + L-dopa, 50 mg kg^{-1}	8	162·75 ± 16·43	495·0 ± 5·00	380· ± 37·79	303·75 ± 33·95

There was no significant differences between 3-MBA alone and 3-MBA + naloxone or L-dopa.

& Spring (1983), DMSO produced a small, but statistically significant, analgesic effect. Clearly, 3-MBA produced significant effects in the analgesia test in a dose- and time-related manner.

As shown in Table 1, neither naloxone nor L-dopa affected the ability of 3-MBA to elevate the threshold for response to pain in the analgesia test.

Discussion

These data clearly show that 3-MBA produces a dose-dependent elevation of the pain threshold in rats. Attempts to gain some insight into the mechanism of the analgesic effect of this benzamide were unsuccessful. Several other substituted benzamides have dopamine receptor blocking activity and at least one (metoclopramide) has analgesic properties (Ramaswamy & Bapna 1986). The results of that suggest involvement of both opiate and dopaminergic receptors in the mechanism of the analgesic effects of metoclopramide. Since opiate receptors are clearly involved in pain regulatory mechanisms, the effects of the opiate receptor antagonist naloxone and the dopamine precursor, L-dopa on 3-MBA analgesia were tested. No evidence was obtained for involvement of either of these receptors, in that neither naloxone (0·5 mg kg^{-1}) nor L-dopa (50 mg kg^{-1}) significantly altered the analgesic response.

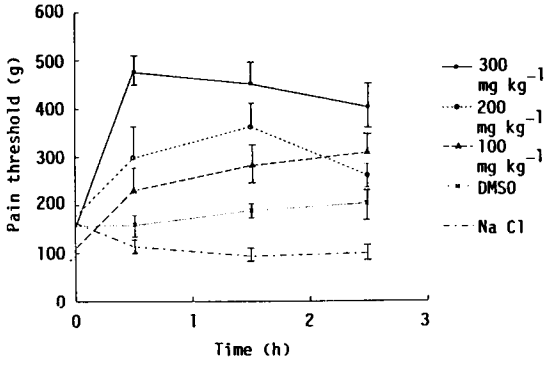

FIG. 1. Pain threshold of rats as determined by the paw pinch test at three time intervals following i.p. injection of the following amounts of drugs: × equal volume of DMSO; △ 100 mg kg^{-1} 3-MBA; ○ 200 mg kg^{-1} 3-MBA; ● 300 mg kg^{-1} 3-MBA; –·–·– Saline. DMSO was the solvent for 3-MBA. Saline served as a control for DMSO. 3-MBA differed significantly from DMSO as follows: 100 mg kg^{-1} at 1·5 h, $P < 0·05$; 200 mg kg^{-1} at 1·5 h, $P < 0·01$; 300 mg kg^{-1} at 0·5 h and 1·5 h, $P < 0·001$, and 2·5 h, $P < 0·01$. Saline and DMSO differed at 1·5 h, $P < 0·001$ and at 2·5 h $P < 0·01$. Each point represent the mean obtained from 8 rats ± s.e.

The dose of naloxone we used was less than the 5 mg kg^{-1} used by Ramaswamy & Bapna (1986), but metoclopramide is substantially more potent as an analgesic than 3-MBA. Additionally, Smits & Takemori (1970), in a study of the antagonism of narcotics by naloxone, showed that as little as 0·04 mg kg^{-1} naloxone would effectively reverse the analgesic effects of 0·5 mg kg^{-1} morphine sulphate in mice. Thus, it might be expected that the dose of naloxone used herein would have an effect on the 3-MBA-induced analgesia if opiate receptors were operative in its mechanism of action. However, this single observation should be taken as tentative.

The amounts of L-dopa and carbidopa used elevate brain levels of dopamine and produce behavioral changes in mice were shown by Goodale & Moore (1976). If dophaminergic mechanisms were operative in the 3-MBA effects, some change in the 3-MBA-induced analgesia in the L-dopa treated animals would also be expected, but as with naloxone, none were seen. Thus, 3-MBAs analgesic activity, does not appear to be associated with opiate or dopamine receptors.

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