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# Is there a simple explanation for the sensitization to histamine produced by adrenergic β-receptor antagonists?

The high resistance of mice to the lethal effects of histamine can be reduced by *Bacillus pertussis* vaccine (Parfentjev & Goodline, 1948; Munoz & Bergman, 1966) or by adrenergic β-receptor antagonists (Fishel, Szentivanyl & Talamage, 1962; Townley, Trapani & Szentivanyl, 1967; Bergman & Munoz, 1968). On this basis, Fishel, Szentivanyl & Talamage (1964) have argued that the lethal effects of histamine in mice are usually antagonized by the simultaneous release of large amounts of catecholamines; when *B. pertussis* vaccine or other drugs block the adrenergic β-receptors, lethality is believed to have been increased by unbalanced α-receptor stimulation. This explanation is vulnerable because it has also been shown that histamine lethality is enhanced by adrenalectomy (Halpern & Wood, 1950) when a reduction in both α- and β-receptor stimulation would be expected, and that the enhanced lethality produced by a β-receptor antagonist can be reversed by a large dose of adrenaline (Bergman & Munoz, 1966, 1968) when even greater α-receptor stimulation would be expected. There is, of course, a simpler explanation which would resolve these difficulties. It is conceivable that histamine produces death in mice by acute bronchoconstriction and that this action is usually attenuated by the bronchodilator action of histamine-released catecholamines. Before attempting to evaluate this hypothesis directly, it seemed important to assess the potential effects of the non-specific actions of adrenergic β-receptor antagonists. To do this, the effects of both (+)- and (−)-isomers of D-(−)-2-isopropylamino-1-(*p*-nitrophenyl)ethanol (INPEA) (Almirante & Murmann, 1966) have been studied.

Differences in sensitivity to histamine due to environmental conditions and strain differences are known to exist. For this investigation, therefore, 2 random-bred strains (NMRI and AP-1) and 5 inbred strains (C3H/He/Sel, CE/Sel, DBA/2/Sel, C57L/Sel, C57Bl/10/Sel) were used with the aim of finding the most suitable strain. All the animals, including the inbred strains, come from our breeding station where they were kept solely on a diet of Rieper/MT pellets and deionized water. The environmental conditions in the laboratory were the same as in the animal quarters ($25° \pm 0{\cdot}3$). The animals had been fasted for 18 h before testing. To measure the sensitivity to histamine, groups of 10 adult male mice of each strain were given, intraperitoneally, doses of histamine HCl in saline corresponding to 15, 60 and 600 mg/kg histamine base. D-(−)-INPEA, L-(+)-INPEA and propranolol were injected intravenously at various doses in 0·2 ml saline at the rate of 0·01 ml/s, 15 min before the histamine challenge. Mice sensitive to histamine showed sedation, cyanosis, defeacation, unsteady gait and respiratory distress. Many of these animals convulsed and died within 5–20 min of the challenge injection. Only the 24 h toxicity value of histamine was estimated.

All the strains used showed the usual high resistance to the lethal effects of histamine and different animals in any strain varied greatly, both in the effects produced by

histamine and their sensitivity to the drug. Thus, no clear dose-activity relation could be established in any strain. In fact, the percentage of control animals that died after a challenge with 60 mg/kg was not significantly different from that observed after a challenge with 600 mg/kg. In 6 out of 7 strains, the control lethality observed was always between 0–10%.

In an attempt to investigate the dose-activity relations of the agents under examination in a first series of experiments, groups of NMRI mice were challenged with standard doses of histamine, corresponding to 15, 60 and 600 mg/kg histamine base, respectively, 15 min after treatment with graded doses of the agents under examination or saline. The results obtained are reported in Table 1.

Table 1. *Effects of* D-(−)- *and* L-(+)-INPEA *on histamine toxicity in NMRI mice*

| Dose | Histamine (base) challenge 15 mg/kg i.p. | | | Histamine (base) challenge 60 mg/kg i.p. | | | Histamine (base) challenge 600 mg/kg i.p. | | |
|---|---|---|---|---|---|---|---|---|---|
| mg/kg i.v. | D-(−)-INPEA | L-(+)-INPEA | Propranolol | D-(−)-INPEA | L-(+)-INPEA | Propranolol | D-(−)-INPEA | L-(+)-INPEA | Propranolol |
| 1 | 0/20* 0 | 2/20 10 | 4/30 13 | 10/20 50 | 1/20 5 | 9/20 45 | 6/20 30 | — | 3/30 10 |
| 6 | 2/20 10 | 2/20 10 | 8/20 40 | 8/20 40 | 1/20 5 | 9/20 45 | 12/30 40 | 14/30 47 | 18/30 60 |
| 12 | 3/20 15 | 2/20 10 | 12/20 60 | 13/20 65 | 2/20 10 | 16/20 80 | 16/40 40 | 8/40 20 | 16/30 53 |
| 24 | 7/20 35 | 2/20 10 | — | 14/20 70 | 2/20 10 | — | 19/40 48 | 21/40 53 | 24/30 80 |
| 48 | — | — | — | — | — | — | 25/30 83 | 27/30 90 | — |
| Saline | | 1/50 5 | | | 4/50 8 | | | 4/40 10 | |

* Death/number and percentage of death of mice tested.

Table 2. *Effects of* D-(−)- *and* L-(+)-INPEA *on histamine toxicity in 6 different strains of mice*

| Histamine (base) challenge mg/kg i.p. | Agent | Dose mg/kg i.v. | AP-1* | C3H/He/Sel | CE/Sel | DBA/2/Sel | C57L/Sel | C57B1/10/Sel |
|---|---|---|---|---|---|---|---|---|
| 60 | D-(−)-INPEA | 12 | 4/10† 40 | 3/10 30 | 0/10 0 | 0/20 0 | 4/7 57 | 2/10 20 |
| | L-(+)-INPEA | 12 | 5/10 50 | 1/10 10 | 0/10 0 | 0/20 0 | 1/7 14 | 0/10 0 |
| | Propranolol | 6 | 9/10 90 | 4/10 40 | 3/10 30 | 3/20 15 | 7/7 100 | 4/10 40 |
| | Saline | — | 0/10 0 | 0/10 0 | 0/10 0 | 0/20 0 | 0/7 0 | 0/10 0 |
| 600 | D-(−)-INPEA | 12 | 8/10 80 | 4/10 40 | 7/10 70 | 7/10 70 | 7/7 100 | 3/10 30 |
| | L-(+)-INPEA | 12 | 8/10 80 | 0/10 0 | 8/10 80 | 6/10 60 | 5/7 72 | 1/10 10 |
| | Propranolol | 6 | 7/10 70 | 3/10 30 | 6/10 60 | 4/10 40 | 7/7 100 | 4/10 40 |
| | Saline | — | 1/10 10 | 1/10 10 | 0/10 0 | 1/10 10 | 2/7 29 | 0/10 0 |

* Strain used.
† Death/number and percentage of death of mice tested.

In the NMRI mice challenged with 15 and 60 mg/kg of histamine, both propranolol, and to a somewhat lesser extent D-(−)-INPEA, enhanced the lethal effects of histamine, while L-(+)-INPEA was without effect. After challenge with 600 mg/kg of histamine, on the other hand, all three agents markedly potentiated histamine toxicity. It is apparent that the mechanism of the sensitizing action observed after challenge with 15 and 60 mg/kg histamine differs from that seen after challenge with 600 mg/kg. These observations are compatible with the assumption that the effect seen after the lower challenges is due to β-adrenergic receptor blockade; the effect seen in the highly challenged mice, on the other hand, does not appear to have such a simple explanation.

In a second series of experiments, groups of male mice of 6 different strains were challenged with two doses of histamine, i.e. 60 and 600 mg/kg (histamine base), respectively, 15 min after injecting a standard dose of the test agents. The results obtained are in Table 2.

Some interesting patterns emerged from this investigation. As was found with NMRI mice, histamine alone was not more toxic to mice of any strain (except perhaps strain C57L/Sel) after 600 mg/kg than after 60 mg/kg intraperitoneally. However, evidence of strain differences to histamine sensitization was found, especially in the mice challenged with 80 mg/kg of histamine. Strain C57L/Sel seemed to be most sensitive to histamine potentiation by β-adrenergic blockade, but the other

inbred strains too, showed some histamine sensitization after propranolol. The behaviour of D-(−)-INPEA in these experiments perhaps could be explained by the lower adrenergic β-blocking potency of this agent compared with that of propranolol. L-(+)-INPEA, again, was ineffective. Random-bred strain AP-1, however, after challenge with 80 mg/kg of histamine, behaved in exactly the same manner as observed after challenge with 600 mg/kg, indicating a particular sensitivity to histamine sensitization. In all other strains, and unlike the response to 60 mg/kg of histamine, all three agents produced about the same toxic effect after 600 mg/kg histamine. No significant difference could be seen between the enhancing effect of the β-blockers D-(−)-INPEA and propranolol and that of the non-blocking isomer L-(+)-INPEA. There is thus a suggestion of a completely different mechanism of action.

Some conclusions concerning the mechanism of action can be drawn. In the mice challenged with the low doses of histamine, the behaviour of D-(−)-INPEA runs parallel with that of propranolol. Consequently, the same interpretation could apply; that there is a good correlation between β-receptor blockade and sensitization to histamine toxicity. Nevertheless, it is puzzling that although D-(−)-INPEA and propranolol were given at doses causing highly effective blockade of β-adrenergic receptors, in only one strain (C57L/Sel) was a 100% mortality to the challenge of histamine observed. This is at variance with the results of Bergman & Munoz (1968) who still observed 100% lethality in their CFW mice with doses of propranolol much lower than those we used, and this could mean that the same individual differences in the sensitivity to histamine might also exist for the histamine-sensitizing effect of other drugs. This would indicate that even complete β-blockade is not capable of sufficiently sensitizing those animals that are particularly resistant to histamine. Strain C57L/Sel on the other hand, might be particularly sensitive to histamine and consequently give results more like those described by Bergman & Munoz (1968) with CFW mice.

The mechanism by which sensitivity of pretreated mice to very high doses of histamine was raised is not clear. Since D-(−)- as well as L-(+)-INPEA were equally effective, the sensitization to histamine toxicity cannot be explained by actions on the adrenergic β-receptors. These data indicate clearly that at high histamine challenge the interference would be unspecific. Again, it is interesting that even with massive doses of histamine only occasionally was 100% lethality observed.

In conclusion, it seems unlikely that a simple explanation can be given at present to explain the effects of drugs in increasing the lethality to histamine.

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