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ANTIPHLOGISTIC ACTIVITY OF IPRONIAZID

BY

I. SETNIKAR, M. SALVATERRA AND O. TEMELCOU

From the Research Department, Recordati Laboratorio Farmacologico, Milan, Italy

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Iproniazid was found to inhibit formalin-induced oedema of the foot, dextran-induced oedema and cotton-pellet-induced granulomatous tissue in the rat. The presence of the adrenals was essential for the antiphlogistic activity. The antiphlogistic action was not accompanied by any antipyretic effect. Inhibition of formalin-induced oedema was also observed with phenylbutazone and with salicylamide.

Little is known about the therapeutic efficacy and mechanism of action of iproniazid in rheumatoid arthritis. In the opinion of Scherbel (1957, 1958a and b), the main effect of the drug is to improve the psychic condition of patients. However, Banghart (1958), using higher doses, frequently obtained objective improvement, but he also observed side-effects in a large number of patients and frequently had to reduce the dose or even suspend treatment with the drug.

This paper describes a pharmacological investigation of the mechanism of action of iproniazid in artificially induced inflammatory states.

METHODS

Antiphlogistic Action

Formaldehyde-induced Oedema. — Oedema was induced in the hind foot by the injection of 0.1 ml. of a 3% solution of formaldehyde into the plantar aponeurosis of male rats (150 to 250 g.). The increase in the dorsoplantar thickness of the injected foot was determined at hourly intervals for 6 hr. by a calliper rule applied at a level between the two proximal and the two medial plantar eminences. With a little experience the calliper jaws could be applied at a constant pressure and the measurements of thickness were reproducible within ± 0.1 mm. Drugs were injected subcutaneously immediately before the injection of formaldehyde.

Dextran-induced Oedema.—Oedema was induced by the intraperitoneal injection of 300 mg./kg. of dextran into male rats (150 to 250 g.). Oedema in the hind feet was measured as described above. Iproniazid was injected subcutaneously 30 min. before the administration of dextran.

Cotton-pellet-induced Granulomata.—The weights of the granulomata, which formed after the subcutaneous insertion of cotton pellets weighing 18 to 19 mg. into male rats weighing 70 to 80 g., were estimated by the method of Meier, Schuler, and Desaulles (1950). Administration of iproniazid was started the day after implantation, and either 50 or 100 mg./kg. of the drug was given subcutaneously daily for six days. On the eighth day after implantation the rats were killed, the granulomata removed, freed from the cotton nucleus and wet-weighed.

Antipyretic Action

This was tested by the subcutaneous injection of the drugs into rabbits which received immediately afterwards an intravenous injection of 1 ml. of anti-typhoid vaccine mixed with 1 ml. of a 20% suspension of brewers' yeast. Rectal temperatures were measured by thermocouples.

RESULTS

Formaldehyde-induced Oedema. — Iproniazid in doses of 200 mg./kg. almost completely inhibited a formaldehyde-induced oedema (Fig. 1). With doses of 100 and 50 mg./kg., the inhibition was less marked but statistically significant. The intensity of the antiphlogistic action was expressed quantitatively by measuring the area enclosed between the curves for the control and treated animals (Figs. 1 and 2). The logarithm of the dose and the surface (expressed in arbitrary units) of these areas are clearly proportional (Fig. 3).

The antiphlogistic effects of phenylbutazone and salicylamide are represented in Fig. 2. It was not possible to calculate a potency ratio between iproniazid and phenylbutazone because the slopes of the dose/activity lines are different. However, iproniazid seemed to be somewhat more active than phenylbutazone and far more active than salicylamide with this test. Moreover, since the intraperitoneal LD50 of iproniazid was 382 mg./kg. (range 456 to 320), while that of phenylbutazone was 266 mg./kg. (284 to 248), using the

method of Litchfield and Wilcoxon (1949), the therapeutic index of iproniazid seems to be decidedly better than that of phenylbutazone.

Dextran-induced Oedema.—The inhibition of dextran-induced oedema by iproniazid seemed to be less intense and more fleeting than that of oedema induced by formaldehyde (Fig. 4). Indeed, at the end of the second hour there was no statistically significant difference between the control and treated rats.

Cotton-pellet-induced Granulomata.—The administration of both 50 and 100 mg. of iproniazid/kg. for six days led to a decrease in the weight of foreign body granulomata which was both statistically significant and proportional to the dose (Table I). Control measurements of body weight and of the weight of various internal organs showed a decrease in the weight of the thymus, though this was only statistically significant after the daily dose of 100 mg./kg.

Adrenal Function.—To check the effects of iproniazid upon adrenal function 50 mg./kg. of the drug in three equal doses was given subcutaneously to rats of both sexes with body weights of 140 to 160 g. After 24 hr., no change in the weight of the adrenals could be detected nor any alteration in their ascorbic acid and cholesterol contents as compared with the controls. This applied both to starved rats and to rats subjected to immobilization stress (Setnikar and Murmann, 1958). The thymus weight always

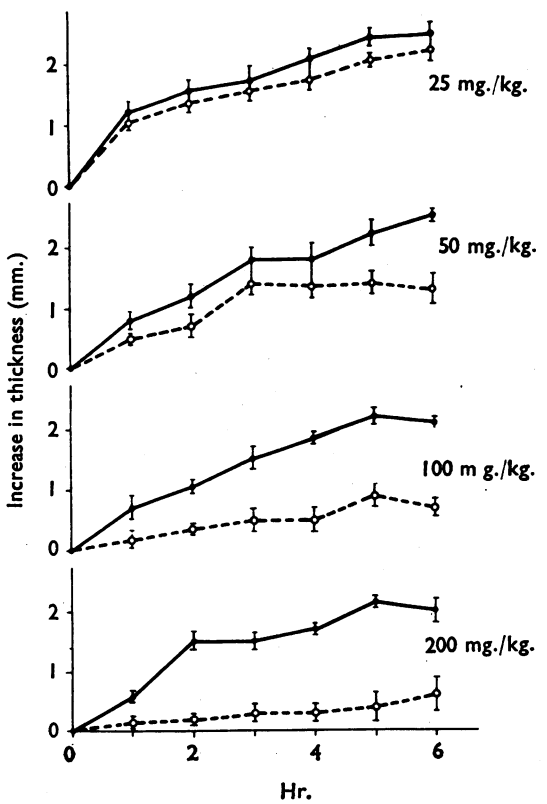

FIG. 1.—Inhibition of the increase in thickness of the hind feet of rats caused by the injection of formaldehyde with graded subcutaneous doses of iproniazid. Every dose was tested on ten rats and there were ten controls. Statistically significant differences between treated rats and controls are observed with doses of iproniazid of 50 mg./kg. and over. Solid lines, controls; broken lines, treated rats.

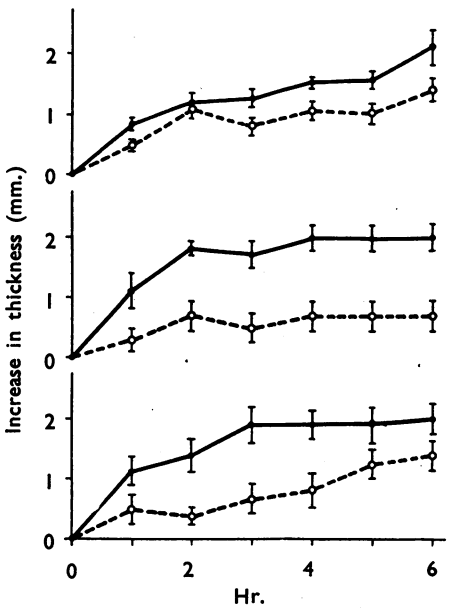

FIG. 2.—Inhibition of formalin-induced plantar oedema by phenylbutazone and salicylamide given subcutaneously. Solid lines, controls; broken lines, treated rats. Upper curves, phenylbutazone, 135 mg./kg.; middle curves, phenylbutazone, 200 mg./kg.; lowest curves, salicylamide, 500 mg./kg.

TABLE I

INHIBITION OF GRANULOMA FORMATION BY IPRONIAZID

Daily Dose of Iproniazid (mg./kg.)	No. of Rats	No. of Granulomata	Average Weight of Granulomata (mg.)	Difference between Controls and Treated Rats	Probability of Difference (*P*)
0	10	40	167±2		
50	10	40	153±3	−8%	<0·001
0	10	40	162±5		
100	10	40	143±2	−12%	<0·001

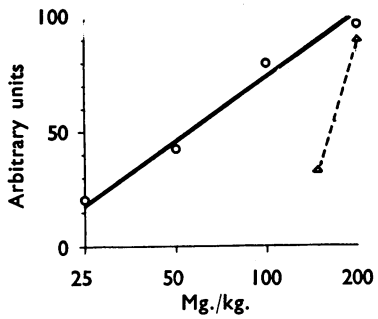

FIG. 3.—Antiphlogistic effect of iproniazid (solid line) and phenylbutazone (broken line). Ordinate: arbitrary units measuring the areas enclosed by the curves for treated animals and controls in Figs. 1 and 2. Abscissa: logarithm of the dose. Note that the slopes of the activity/log dose lines are very different for the two drugs.

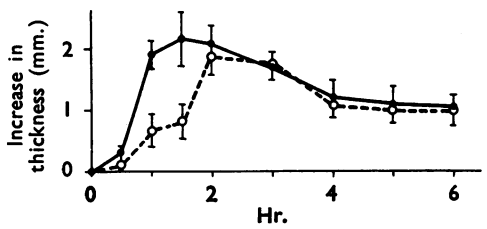

FIG. 4.—Effect of 100 mg./kg. of iproniazid (broken line) on the increase in dorso-plantar thickness of the hind feet of rats to which 300 mg./kg. of dextran had been given intraperitoneally. Maximum oedema occurred 90 min. after injection. The maximum difference between controls (solid line) and animals treated with iproniazid can be observed during the same period. Each point is the average value from 14 rats.

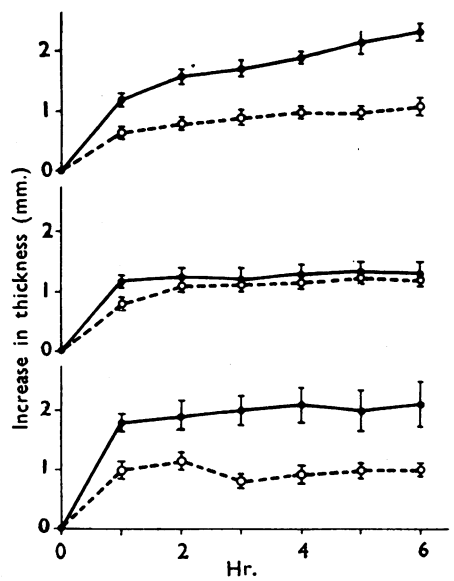

FIG. 5.—Effects of 100 mg./kg. of iproniazid (broken lines) on plantar oedema induced by formaldehyde in normal (upper curves), adrenalectomized (middle curves), and hypophysectomized (lower curves) rats.

showed a decrease, but this was not statistically significant.

Formaldehyde-induced Oedema in Adrenalectomized and Hypophysectomized Rats.—Iproniazid (100 mg./kg. subcutaneously) exerted a clear antiphlogistic effect in hypophysectomized rats, comparable to that in normal animals, but after adrenalectomy the drug had practically no action; only after the first hour was there a small, but statistically significant, difference ($0.02<P<0.05$) between treated rats and controls (Fig. 5). It seems therefore that the adrenals are necessary for the antiphlogistic activity of iproniazid.

Antipyretic Action.—Iproniazid up to 200 mg./kg. subcutaneously exerted no antipyretic action in hyperthermal rabbits, whereas the action of phenylbutazone was evident with 100 mg./kg. doses.

DISCUSSION

The antiphlogistic action of iproniazid in doses of 50 mg./kg. or more may explain the antirheumatic activity which, according to Scherbel (1957, 1958a and b) and Banghart (1958), follows large and toxic doses of this drug.

Analogous antiphlogistic effects are observed with similar substances such as isoniazid (Theobald, 1955). For isoniazid too it has been noted that the antiphlogistic action is effective in hypophysectomized animals and is much less in adrenalectomized animals (Domenjoz, 1954).

The hypothesis might be advanced that iproniazid acts by inhibiting the degradation of 5-hydroxytryptamine, which also has an antiphlogistic effect (Georges and Herold, 1957). On the other hand, while both iproniazid and 5-hydroxytryptamine act only in the presence of the adrenals, the former does not seem to affect either normal or stress-stimulated adrenal activity, whereas the latter has an antagonistic effect upon the reduction of ascorbic acid caused by stress (Georges and Herold, 1958; Georges, 1957). It is therefore possible that the antiphlogistic action of 5-hydroxytryptamine is due to a modification of the hormonal secretion of the adrenals while that of iproniazid may be due to another mechanism, for example, an inhibition of hepatic degradation of adrenal antiphlogistic hormones, a mode of action which has already been invoked to explain the antiphlogistic action of isoniazid (Wiesel, 1956; Wiesel, Barritt and Scheid, 1956).

In conclusion, some of the antirheumatic effects of large doses of iproniazid in man seen by Scherbel (1957, 1958a and b) and by

Banghart (1958) may be explained by the antiphlogistic action. It is therefore probable that, in rheumatoid arthritis, iproniazid exerts a direct therapeutic action on the lesions and not merely an indirect action, namely an improvement in psychic condition as suggested by Scherbel (1958a and b). But it must be admitted that this direct therapeutic effect can be obtained only with very high toxic doses of the drug which limits its therapeutic usefulness.

References

Banghart, H. (1958). *J. clin. exp. Psychopath.*, **19**, Suppl. 1, 114.

Domenjoz, R. (1954). *Actualités pharmacol.*, **7**, 73.

Georges, G. (1957). *C.R. Soc. Biol., Paris*, **151**, 692.

—— and Herold, M. (1957). Ibid., **151**, 695.

—— —— (1958). Ibid., **152**, 436.

Litchfield, J. T., and Wilcoxon, F. (1949). *J. Pharmacol. exp. Ther.*, **96**, 99.

Meier, R., Schuler, W., and Desaulles, P. (1950). *Experientia*, **6**, 469.

Scherbel, A. L. (1957). *Cleveland Clin. Quart.*, **24**, 90.

—— (1958a). *J. clin. exp. Psychopath.*, **19**, Suppl. 1, 118.

—— (1958b). *J. belge Méd. phys. Rheum.*, **13**, 135.

Setnikar, I., and Murmann, W. (1958). *Farmaco, Ed. sci.*, **13**, 541.

Theobald, W. (1955). *Arch. int. Pharmacodyn.*, **103**, 17.

Wiesel, L. L. (1956). *Amer. J. med. Sci.*, **232**, 412.

—— Barritt, A. S., and Scheid, C. J. (1956). Ibid., **232**, 415.