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Taloximine, a new respiratory stimulant with bronchodilator properties

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1. A novel phthalazine analogue taloximine (1-hydroxyimino-4(2-dimethylaminoethoxy)-1,2-dihydrophthalazine monohydrochloride monohydrate) stimulated respiration in conscious rabbits at doses of 7 mg/kg and above.

2. Taloximine antagonized the depressant action of morphine on respiration in rabbits at doses of 10 mg/kg. At high doses it resuscitated rabbits after they had been given lethal doses of sodium pentobarbitone.

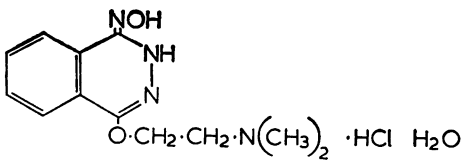
3. In *in vitro* preparations of the trachea or bronchus taloximine was about equiactive with aminophylline. In the Konzett-Rössler preparation the intravenous ED50 for taloximine was about 18% of that of aminophylline, whereas after oral administration the two drugs were equiactive.

4. Taloximine, unlike aminophylline, did not protect guinea-pigs against anaphylactic microshock to egg albumen.

5. Taloximine shortened the duration of the loss of righting reflex in mice due to hexobarbitone more effectively than bemegride, nikethamide or vanillic acid diethylamide.

6. In the dose required to stimulate respiration taloximine had only slight cardiovascular effects. Up to four times this dose produced no evidence of general excitation of the central nervous system.

The object of this paper is to describe the pharmacology of a new drug for the treatment of acute and chronic respiratory distress, 1-hydroxyimino-4(2-dimethylaminoethoxy)-1,2-dihydrophthalazine monohydrochloride monohydrate (approved name taloximine), one of a series of novel structures based on the phthalazine nucleus (Parsons & Turner, 1967). Experiments are described in which the effects of taloximine on respiration were compared with those of bemegride, nikethamide and vanillic acid diethylamide. The bronchodilator activity of taloximine was also compared with that of aminophylline.



Methods

Respiratory minute volume

Comparative minute volumes were measured in conscious Dutch rabbits by the method of Lightowler & Wilder Smith (1963). Minute volumes at various times after drug injection were expressed as the percentage changes from the resting level.

Antagonism of the depressant action of morphine on respiration

Seven groups of seven Dutch rabbits (1.5–2 kg) received varying doses of taloximine or bemegride intravenously 12 min after the injection of morphine 10 mg/kg. Recordings of respiration were taken 1 min after morphine administration and then at frequent intervals up to 30 min. The results were assessed by plotting the minute volume against time and weighing the area of the paper delineated by the curve and a horizontal line drawn through the resting level. The " respiratory index " was obtained by subtracting areas below the resting value from areas above it. Seven such values were obtained at each dose level of taloximine and bemegride and their means plotted against the dose.

Resuscitation after lethal doses of sodium pentobarbitone

Thirty-three Dutch and four Old English rabbits (1.4–2.1 kg) were used. The minimum lethal dose of sodium pentobarbitone (65 mg/kg) (Fitch & McCandless, 1931) or twice or four times this dose was given intraperitoneally ; 5 min later taloximine was given either by single intravenous injections of 25, 50 or 100 mg/kg or as an infusion of a 1–2% solution at a rate of 5 or 10 mg/min into the marginal ear vein. A solution of 0.9% NaCl was used as a control.

Antagonism of the loss of righting reflex due to hexobarbitone

One hundred and eighty male T.O. mice (14–20 g) were used in groups of ten. Ten minutes after intraperitoneal injection of sodium hexobarbitone (100 mg/kg), solutions of the test compounds in varying concentrations were infused into the tail vein at a rate of 0.53 ml./min for 5 min or until the righting reflex was restored, whichever was the shorter period.

Pentylenetetrazol-induced convulsions

The effects of taloximine on pentylenetetrazol-induced convulsions were investigated as described by Lightowler & MacLean (1963).

Guinea-pig isolated trachea

The direct effects of taloximine and aminophylline were studied on an open spiral chain preparation obtained by cutting the trachea transversely several times from alternate sides. The tracheal chain was suspended in a 10 ml. bath containing Krebs-Henseleit solution at 37° C, gassed with 95% oxygen and 5% carbon dioxide. Acetylcholine (0.15–1.0 μg/ml.) was added to the bath for 2 min to restore the tone of the preparation. Drugs were added to the bath every 15 min and left in contact for 2 min. Seven 16-point analyses of variance were performed.

Human isolated bronchus

The material was obtained during operation for bronchial carcinoma and was dissected from apparently normal tissue adjacent to the tumour. It was placed immediately in ice-cold Krebs-Henseleit solution and kept for up to 24 hr. Strips were linked together with cotton to form a preparation 5 cm. long (expt. 1) or cut into three complete rings tied end to end with cotton to form a preparation 5.9 cm long (expt. 2). The preparations were suspended in a 25 ml. bath containing Krebs-Henseleit solution at 37° C, gassed with 95% oxygen and 5% carbon dioxide. Constant sub-maximal responses to acetylcholine (4 μg/ml.) were obtained at 20 min intervals with a 4 min contact time ; taloximine or aminophylline were administered 4 min before the standard dose of acetylcholine, which was added without washout. After relaxation by taloximine the tone of the tissue was restored by several washouts and repeated additions of the standard dose of acetylcholine. A solution of 0.9% NaCl was used as control.

Inhibition of histamine-induced bronchoconstriction in the anaesthetized guinea-pig

The preparation was as described by Konzett & Rössler (1940). Male guinea-pigs (260–540 g) were anaesthetized by intraperitoneal injection of a dose of allobarbitone which was sufficient to suppress spontaneous respiration. Bronchoconstriction was produced by intravenous injection of histamine (1–6 μg) at intervals of 6 min.

In the experiments with intravenous injection of taloximine or aminophylline, the guinea-pigs were first given an injection of heparin (1,000 i.u./kg) into the right external jugular vein. Solutions of drugs (maximum volume 0.5 ml.) were injected and washed in with 0.4 ml. of 0.9% NaCl solution immediately before a standard dose of histamine. Drug doses (ED50) that gave 50% inhibition of the standard histamine response were calculated. Taloximine was given before aminophylline in four assays and aminophylline before taloximine in three assays.

When the drugs were given orally, a catheter was inserted into the stomach through the mouth. Four constant responses to intravenous injection of histamine were obtained before the first oral dose of 0.9% NaCl solution. Solutions of 0.9% NaCl, taloximine or aminophylline (37° C) were given over a 5 min period. Dose volumes were 1 ml./100 g body weight and washed in with 1 ml. saline. Histamine was given intravenously every 6 min for 1 hr after the first control dose of 0.9% NaCl solution and then for 3 hr after the drug or second 0.9% NaCl control dose. The percentage reduction of the histamine response was calculated from the depression of the response obtained 6 min after administration of taloximine or aminophylline, the mean of the four responses preceding drug administration being taken as 100.

Protective effect against anaphylactic microshock in the guinea-pig

Taloximine was compared with aminophylline for the ability to relieve the respiratory distress of sensitized guinea-pigs to an aerosol of egg albumen. The method of Herxheimer (1952) and Herxheimer & Rosa (1953) as modified by Smith (1961) was used.

The number of seconds between exposure of the guinea-pigs to the albumen aerosol and the onset of severe dyspnoea was recorded. The animals were quickly

removed from the chamber at this point. The duration of exposure to the aerosol after treatment divided by the mean duration of exposure on the seventh day before and on the seventh day after the test gave the protection ratio.

Cardiovascular studies

The effects on blood pressure and heart rate were studied in cats anaesthetized with allobarbitone (100 mg/kg) intraperitoneally and in beagles anaesthetized with pentobarbitone sodium (30 mg/kg) intravenously. The effect of taloximine on the e.c.g. (lead II) was studied in anaesthetized beagles in two ways: (*a*) in graded dose experiments taloximine was administered intravenously at 30 min intervals in doses from 0.125 mg/kg to 64 mg/kg, rising in a geometric progression by a factor of 2, 0.9% NaCl solution being given 15 min before each dose and records assessed 30 sec, 1, 2, 3, 4 and 5 min after each dose ; (*b*) in experiments with continuous infusion of taloximine into the femoral vein (0.55 mg/kg per min) e.c.g. records were assessed 1, 5 and 15 min after the start of the infusion and then every 15 min to 180 min.

The isolated heart of the guinea-pig was perfused by the method of Langendorff to measure the effects of taloximine on the heart rate, amplitude of beat and coronary circulation. Filtered Locke solution was used as a control and as solvent for drugs ; it was gassed with oxygen.

Anti-acetylcholine and anti-histamine actions

Guinea-pig ileum. A segment of ileum was suspended in a 10 ml. bath containing Tyrode solution at 32° C, gassed with air. Histamine (0.1–2 μg/ml.) was the agonist. Every 4 min histamine was added to the bath and left in contact with the tissue for 30 sec. Drugs were added 2 min before histamine when the previous three responses to histamine had been constant. Thus the time interval between drugs was variable. Seven 16-point analyses of variance were performed.

Rat duodenum. A segment of duodenum was suspended in a 10 ml. bath containing De Jalon-Ringer solution at 32° C, gassed with air. The relaxations caused by taloximine and aminophylline were recorded. The contact time was 45 sec and a 10 min cycle was maintained. Seven 16-point analyses of variance were performed.

Rat colon. A segment of ascending colon was suspended in a 10 ml. bath containing " rat colon " Ringer solution (Gaddum, Peart & Vogt, 1949) at 32° C, gassed with air. Acetylcholine was the agonist in a suitable submaximal dose. Drugs were added 2 min before acetylcholine when the previous three responses to acetylcholine had been constant. Seven 16-point analyses of variance were performed.

In all isolated tissue experiments the contractions were recorded isotonically by a lever writing on a smoked drum.

Acute toxicity

LD50 values were determined in male and female T.O. strain mice (18–22 g) 24 hr after intravenous, intraperitoneal and oral administration. Ten animals were used in each group and the methods of calculation were those of Litchfield & Wilcoxon (1949) and de Beer (1945).

Drugs

The drugs used were (*a*) allobarbitone (Ciba Laboratories Ltd.); aminophylline (May & Baker Ltd.); bemegride (Nicholas Research Institute); heparin solution (Evans Medical Ltd.); morphine hydrochloride (MacFarlane Smith Ltd.); nikethamide (Ciba Laboratories Ltd.); pentylenetetrazol (Alwitt Ltd.); phenoxybenzamine (Smith Kline & French Laboratories Ltd.) and taloximine (Riker Laboratories, code WG. 109); (*b*) acetylcholine chloride (L. Light & Co. Ltd.); (±)-adrenaline bitartrate (British Drug Houses Ltd.); histamine acid phosphate (Koch-Light Laboratories); (±)-isoprenaline sulphate (Riker Laboratories); lignocaine hydrochloride (Astra-Hewlett Ltd.); (±)-noradrenaline bitartrate (L. Light & Co. Ltd.); pentobarbitone sodium (Abbott Laboratories Ltd.) and vanillic acid diethylamide (Riker Laboratories).

All compounds were dissolved in 0.9% NaCl solution and the pH adjusted with dilute acid or alkali where necessary; the weights refer to the bases (*a*) or the salts (*b*).

Results

Respiratory minute volume

The effect of taloximine on the respiratory minute volume of conscious Dutch rabbits was compared with the effects of bemegride, nikethamide and vanillic acid diethylamide.

Single intravenous injections were given to groups of five rabbits at either three or four dose levels (Fig. 1). The highest doses of bemegride (2 mg/kg) and vanillic acid diethylamide (2 mg/kg) were close to the convulsive threshold and neither of these substances had any effect at one quarter of the convulsive threshold dose (0.5 mg/kg). The dose of taloximine (14 mg/kg) which caused an increase in minute volume of 450% was one quarter of the convulsive dose (60 mg/kg) and increases of 50–150% lasting for at least 10 min were obtained with one eighth of the convulsive dose. The results with nikethamide were intermediate between taloximine and bemegride or vanillic acid diethylamide.

In four experiments with slow intravenous infusion taloximine and bemegride were administered at a rate of 0.85 mg/min (Fig. 2). With taloximine the minute volume increased from the third minute to give an approximately 1,000% increase after 17 min. With bemegride the minute volume started to rise at 1 min and showed an increase of 1,000% after 10 min. Both rabbits given bemegride convulsed at 13 min.

Antagonism of the depressant action of morphine on respiration

Taloximine was as effective an antagonist as bemegride but only at three times the dose (Fig. 3). It was longer acting than bemegride and could be used in doses of 10 mg/kg, whereas bemegride produced convulsions in doses of 2 mg/kg.

Resuscitation after lethal doses of pentobarbitone sodium

After intraperitoneal administration of a lethal dose of pentobarbitone sodium (65 mg/kg) four or five rabbits survived when given taloximine by intravenous infusion at a rate of 5–10 mg/min for a period of 20–22 min. After pentobarbitone

130 mg/kg two of five animals survived when taloximine was infused for 22 min. An infusion for 20 min protected one of five rabbits which had received pentobarbitone sodium 260 mg/kg. Infusions of 0.9% NaCl solution had no protective effect. The rabbits which recovered did not show any alteration in behaviour which distinguished them from normal, untreated animals.

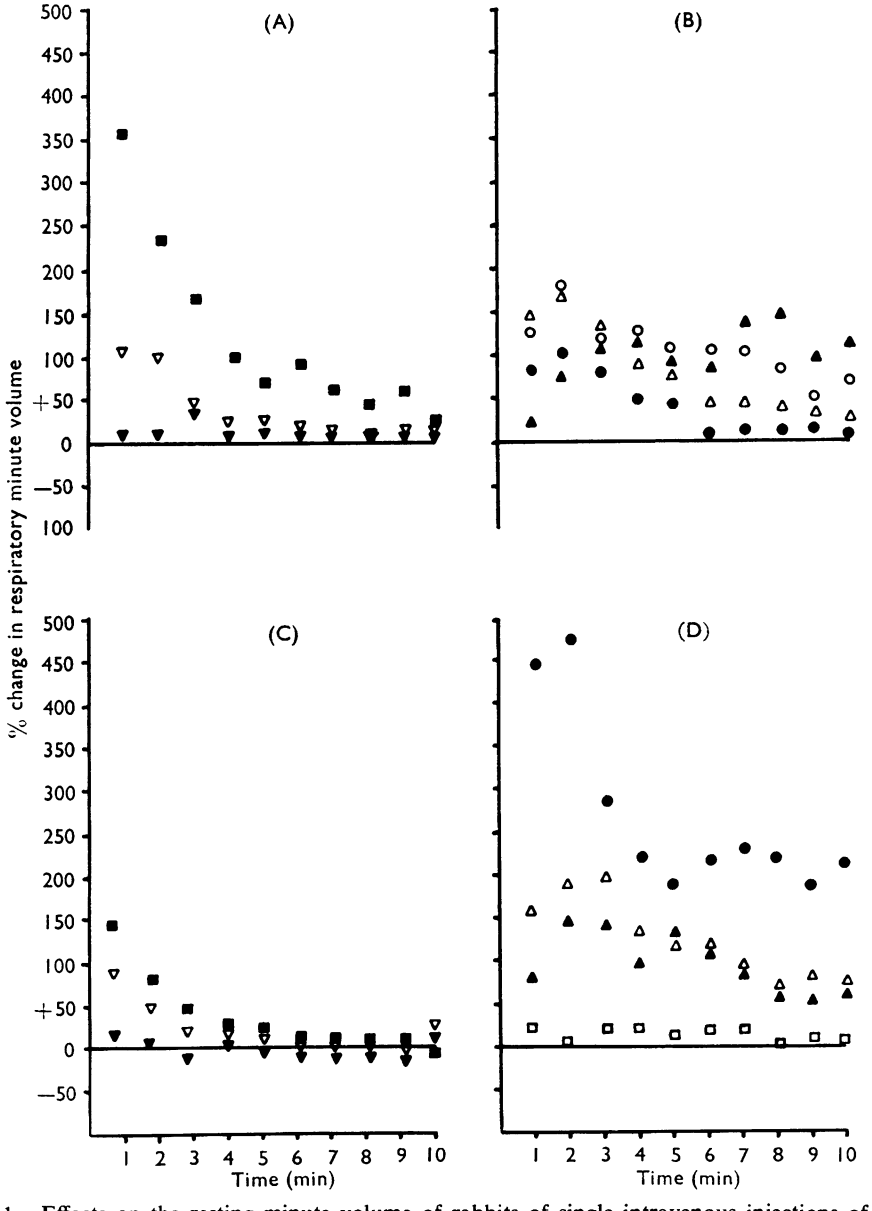


FIG. 1. Effects on the resting minute volume of rabbits of single intravenous injections of (A) bemegride, (B) nikethamide, (C) vanillic acid diethylamide and (D) taloximine at time zero. The dose levels are represented 20 (○), 14 (●), 10 (△), 7 (▲), 5 (□), 2 (■), 1 (▽) and 0.5 (▼) mg/kg.

Single repeated injections of taloximine protected three of five rabbits injected with pentobarbitone sodium 130 mg/kg. The total doses of taloximine given to animals which recovered ranged from 217 to 300 mg/kg. The two animals which died had received 67 and 143 mg taloximine respectively; they survived longer than those which had received 0.9% NaCl solution. In rabbits pretreated with pentobarbitone sodium the tolerance to taloximine was much increased; that is, these animals did not convulse after 300 mg/kg, compared with 60 mg/kg in untreated animals.

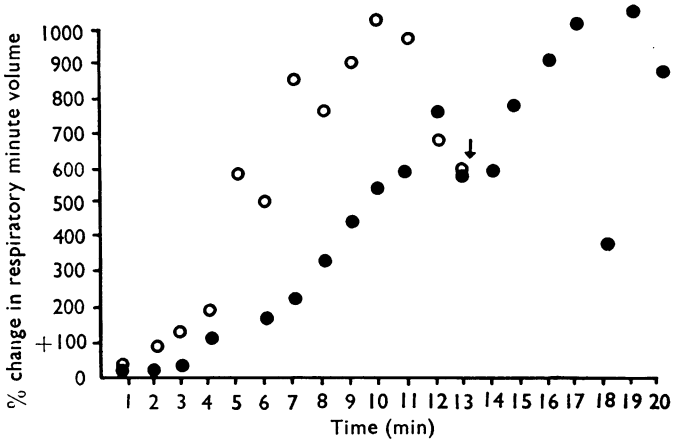


FIG. 2. Effects on the resting minute volume of rabbits of slow infusion into the marginal ear vein of taloximine (●) and bemegride (○) at a rate of 0.85 mg/min from 0 to 20 min. The rabbits given bemegride convulsed at ↓. Each point is the mean of two observations.

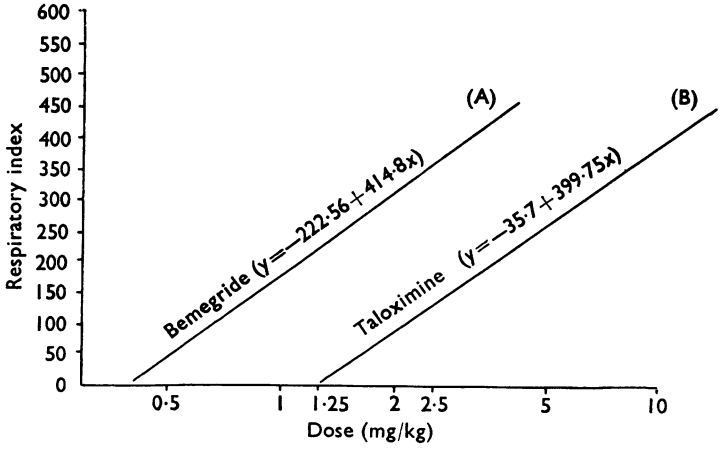


FIG. 3. Relative potencies of bemegride (A) and taloximine (B) in reversing the morphine-induced respiratory depression in rabbits. The bemegride line was calculated from " respiratory indices " of 89.7, 147.9 and 339.2 at corresponding doses of 0.5, 1.0 and 2.0 mg/kg and the taloximine line from " respiratory indices " of 27.8, 161.9, 93.5 and 451.6 at corresponding doses of 1.25, 2.5, 5.0 and 10.0 mg/kg.

Antagonism of the loss of righting reflex due to hexobarbitone

Taloximine had an effect when infused at a rate of 0.05 and 0.025 mg/min. The other substances—nikethamide, bemegride and vanillic acid diethylamide—had an effect at infusion rates of 0.1 mg/min or more (Table 1). The total amount of taloximine that was effective in 5 min was 30 mg/kg or more.

Pentylenetetrazol-induced convulsions

An intravenous injection of pentylenetetrazol (25 mg/kg) stimulated only one animal in a group of ten T.O. strain mice to convulse within 30 sec. Potentiation of this effect, so that five of ten animals convulsed within 30 sec, was obtained with taloximine 95 mg/kg (95% fiducial limits of 72 and 112) or by nikethamide 101 mg/kg (95% fiducial limits of 75 and 134) injected intraperitoneally 5 min before pentylenetetrazol.

Effects on isolated trachea and bronchus

Taloximine was compared with aminophylline on the guinea-pig trachea and human bronchus to assess bronchodilator activity due to relaxation of smooth

TABLE 1. *Restoration of the righting reflex depressed by hexobarbitone*

Compound	Rate of intravenous infusion (mg/min)	Number of mice recovering the righting reflex within 5 min
Taloximine	0·2	8
	0·1	7
	0·05	4
	0·025	4
Nikethamide	0·8	2
	0·4	1
	0·2	0
	0·1	1
Bemegride	0·2	10 (1 convulsed)
	0·1	6
	0·05	0
Vanillic acid diethylamide	0·4	2 (8 died)
	0·2	5
	0·1	0

Relative activities of various respiratory stimulants in restoring the righting reflex after intraperitoneal injection of hexobarbitone (100 mg/kg); each group consisted of ten mice. Control groups of ten animals were injected with 0·9% NaCl solution, but there were no recoveries within 5 min.

TABLE 2. *Relative relaxant activity of taloximine on different isolated organs*

Species	Tissue	No. of estimates	Relative relaxant activity of taloximine (aminophylline = 1·0) S.E. of the mean in parentheses
Guinea-pig	Trachea	7	0·46 (±0·11)
Human	Bronchus (expt. 1)	1	1·2
	Bronchus (expt. 2)	1	0·8–1·6
Guinea-pig	Ileum	7	268 (±16)
Rat	Duodenum	7	9·0 (±2·9)
Rat	Colon	7	14·2 (±1·6)

The relaxant effects were measured either by reduction of the tone of the tissue or the anti-acetylcholine and anti-histamine activities. For details see **Methods**. The results obtained on guinea-pig trachea and ileum and rat colon were assessed statistically by a 16-point analysis of variance.

muscle. Anti-acetylcholine activity was assessed on the rat colon, anti-histamine activity on the guinea-pig ileum and spasmolytic activity on the rat duodenum. The results (Table 2) show that on a weight basis taloximine was less effective than aminophylline in the guinea-pig trachea but not in the other preparations.

Inhibition of histamine-induced bronchoconstriction in the anaesthetized guinea-pig

In seven experiments the inhibitory effects of taloximine and aminophylline were compared after intravenous injections. The ED50 values were 0.53 mg/kg for taloximine and 2.86 mg/kg for aminophylline. Thus, compared with aminophylline, the relative activity of taloximine was 5.4.

When given orally, 0.9% NaCl solution had no effect, whereas in four experiments both taloximine and aminophylline (200 mg/kg) inhibited the histamine-induced bronchoconstriction (Fig. 4). Over the first 48 min after administration the mean inhibition was 89% for aminophylline and 47% for taloximine. When compared with the findings obtained after intravenous administration, these results suggest that after administration to anaesthetized guinea-pigs by stomach tube taloximine was less well absorbed than aminophylline.

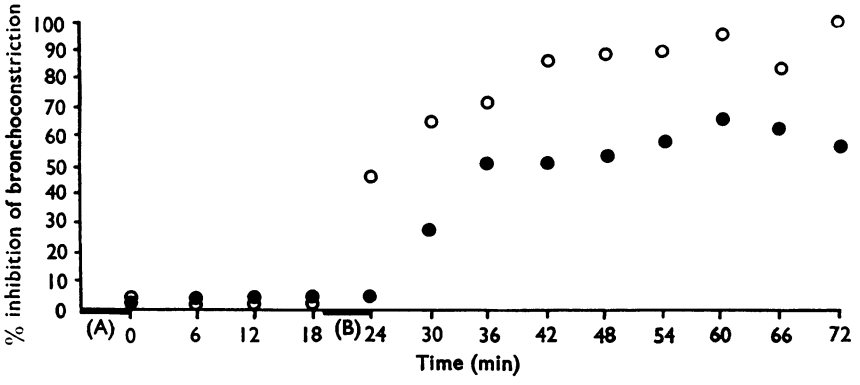


FIG. 4. Comparison of inhibition of histamine-induced bronchoconstriction in the Konzett-Rössler guinea-pig by aminophylline (○) and taloximine (●) given orally over 5 min in a dose of 200 mg/kg in a volume of 1 ml./100 g body weight. 0.9% NaCl solution (1 ml./100 g) at A, drug at B.

TABLE 3. *Anaphylactic microshock in guinea-pigs*

Drug	Dose (mg/kg)	No. of estimations	Mean protective ratio
Taloximine	50	3	1·58
	100	22	1·52
	200	10	6·20
Aminophylline	50	9	3·10
	100	13	7·28
Saline	1 ml./100 g	26	1·12

The protective effects of intraperitoneal injections of taloximine and aminophylline are expressed as the protective ratios as defined in **Methods**.

Protective effect against anaphylactic microshock in the guinea-pig

Taloximine, injected intraperitoneally in doses up to 100 mg/kg 30 min before exposure to an aerosol of the antigen egg albumen, did not protect sensitized guinea-pigs. At 200 mg/kg taloximine gave some protection but the animals were hypersensitive for 1 to 2 hr to external stimuli such as touch and sound. Aminophylline gave protection in doses of 50 and 100 mg/kg (Table 3).

Cardiovascular effects

Cats

In doses of 1 and 2 mg/kg, taloximine caused a transient rise in blood pressure of 40 mm Hg ; doses of 5 and 10 mg/kg caused pressor or biphasic responses and doses of 20 mg/kg and above caused a fall of short duration (1–5 min) of 42 mm Hg or biphasic responses with a small pressor component. The biphasic response to adrenaline (2–12 μg/kg) was unaffected by taloximine 1–5 mg/kg. After 10 mg/kg the depressor component of the response was reduced but the baseline of blood pressure had fallen meanwhile. Responses to noradrenaline (0.4–4 μg/kg) were little affected by taloximine up to 40 mg/kg.

Taloximine in doses of 1, 2, 5 and 10 mg/kg increased the heart rate by 6, 10, 17 and 15 beats per min from 171, 177, 176 and 183 beats/min respectively ; at 20 mg/kg the heart rate slowed by 31 beats/min from 180. The duration of these responses did not exceed 2 min.

In experiments on two cats phenoxybenzamine (10 mg/kg) abolished the response to noradrenaline, converted the pressor response to adrenaline to a depressor response and reduced the rise in blood pressure after taloximine by up to 50%. It is therefore likely that the effect of taloximine on the blood pressure was mediated partly by α-adrenoceptive receptors.

Dogs

When taloximine was administered intravenously in successively increasing doses up to 16 mg/kg the pattern of the e.c.g. remained unchanged. At 32 mg/kg the amplitudes of the QRS complex and T wave were reduced and those of the P wave increased ; the P-R interval was shortened. At 64 mg/kg the amplitude of the QRS complex was further reduced and the P and T wave abolished. Blood pressure changes were confined to variable depressor responses immediately after injection of 16 mg/kg or more. A slight slowing of the heart rate was noted after the higher doses. When taloximine was given by intravenous infusions, at a rate of 0.56 mg/kg per min, the blood pressure fell by less than 10% and there were no consistent changes in the heart rate.

TABLE 4. *Acute toxicity of taloximine in mice*

Route of administration	LD50 mg/kg (with 95% fiducial limits)	
	(*a*)	(*b*)
Intravenous	100 (93–107)	123 (113–134)
Intraperitoneal	203 (193–213)	195 (181–208)
Oral	—	1230 (1100–1530)

(*a*) Method of Litchfield and Wilcoxon (1949), five male and five female mice in each group. (*b*) Method of de Beer (1945), ten male mice in each group.

Guinea-pigs

The effects of taloximine on the guinea-pig isolated heart (fourteen experiments) were expressed as the percentage changes of the heart rate, perfusion rate and amplitude of beat after single doses given directly into the perfusion cannula. As the dose of taloximine was increased the heart rate slowed linearly, from a 10% reduction at 0.5 mg to a 55% reduction at 6 mg. Changes in perfusion rate were dose-dependent and linear from a 15% decrease at 0.2 mg to a 20% increase at 3 mg ; a further increase of the dose produced no greater change in response. There was a transient increase in amplitude of the heart beat lasting less than 1 min ; the maximum of this increase was 50% after single injections of 0.2 mg and 1 mg but 250% after the injection of 6 mg.

Acute toxicity

The LD50 values for taloximine in the mouse are given in Table 4. In rabbits, intravenous administration of less than 35 mg/kg caused no apparent changes in behaviour. After a dose of 60 mg/kg there was slight opisthotonos, increased respiration, tremor and ear twitching lasting 5 min ; after 90 mg/kg death occurred, apparently due to respiratory failure.

Discussion

Respiratory stimulant drugs have been used for their analeptic properties in the treatment of severe respiratory crises with a deeply depressed respiratory centre such as is encountered in morphine and barbiturate poisoning. They have also been used in respiratory failure such as is found in acute exacerbations of chronic bronchitis.

Although a number of compounds are capable of stimulating respiration, few have gained wide clinical acceptance. Their value is limited because the margin between the dose stimulating respiration and the dose causing generalized convulsions is narrow. The most important of the older respiratory stimulants are pentylenetetrazol and nikethamide. The newer drugs, such as bemegride and vanillic acid diethylamide, have been synthesized in the hope that the site of action could be limited to a more specific area in the central nervous system so that the margin of safety would be increased. But they have not been very successful and there is need for a good respiratory stimulant.

Taloximine has been shown to be a powerful stimulant of respiration in untreated conscious rabbits and after depression by morphine. It caused relaxation of the smooth muscle in isolated tracheal and bronchial preparations and inhibited histamine-induced bronchoconstriction. At least four times the effective respiratory stimulant dose was needed to produce any evidence of central excitation and eight times the effective dose to produce convulsions in rabbits. Taloximine also protected rabbits from the effects of lethal doses of pentobarbitone. Doses of about five times the convulsive dose of taloximine were tolerated without convulsions after lethal doses of pentobarbitone, which suggested that the adverse effects of these two substances may have antagonized each other. The fact that taloximine was more effective than other substances in restoring the righting reflex after hexobarbitone and that it had a powerful effect in restoring the respiration after morphine also suggests the possibility of a specific antagonism against central depressant drugs.

Taloximine did not protect sensitized guinea-pigs from anaphylactic respiratory failure. In the dose required to stimulate respiration, taloximine had little effect on the cardiovascular system. The therapeutic safety margin of taloximine appears to be greater than that of nikethamide, bemegride and vanillic acid diethylamide.

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