seed oil base were similar to those with the oil-in-water emulsion in that all three liquids inhibited diffusion. Figure 10 shows the three concentrations of water to significantly retard diffusion. However, the results with water do not differ significantly from each other.

The results with alcohol differ significantly from the control (Fig. 11). As the concentration of alcohol is increased, the retardation of diffusion becomes more pronounced. However, the results with the 2 and 5% do not differ significantly.

The results with DMSO and this base (Fig. 12) are very similar to those obtained with alcohol. All differ significantly from the control. As with the alcohol and this base the difference in results between 2 and 5% DMSO may be taken as due to chance and is not significant. Again as the concentration of liquid is increased inhibition of diffusion becomes more pronounced.

CONCLUSIONS

Undoubtedly the solubility of a drug in an ointment base plays a major part in the diffusion or release of the drug from that base (6). For the most part the solubility of a drug in an intact ointment base is impossible to determine with any degree of confidence due to the fact that one is working with a solid and perhaps a heterogeneous or multiphase system.

Factors which also may influence diffusion in such instances are those of viscosity and the nature of the diffusion membrane. In preliminary experiments alcohol and DMSO in 1, 2, and 5% concentrations did not appreciably influence the passage of salicylic acid from aqueous solutions through the membrane used in this study. From this it is assumed that the liquids used in this study exerted their influence on diffusion by means other than their action on the membrane. Although viscosity measurements were not taken on the ointments, the quantities of liquids added to the various bases were so small that their influence on viscosity would be negligible. If viscosity changes were a great factor in this study, one would expect to get results all going in one direction, probably toward increased diffusion rate, rather than results shown in these experiments, *i.e.*, increased diffusion from two bases and decreased release from the other two.

It is apparent that from this study no general explanation is sufficient to cover all the results. The results indicate that diffusion from ointment bases is greatly influenced by the inclusion of liquids. The characteristics of the base probably determine whether the diffusion process is enhanced or retarded.

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Keyphrases

Salicylic acid diffusion—ointment bases
DMSO, alcohol, water—diffusion effect
Diffusion—ointment base effect
UV spectrophotometry—analysis

Effect of β-Adrenergic Blockade on the Toxicity of Bronchoconstricters in Guinea Pigs

By F. P. LUDUENA and W. B. McKEON, JR.

Propranolol (10 mg./kg.) greatly increased the intravenous toxicity of serotonin, oxotremorine, histamine, and nicotine in guinea pigs. In the case of serotonin, the LD_{50} in the controls was more than 100 times larger than in the propranolol-treated animals. In both groups, bronchoconstriction was the cause of death following the injection of histamine, serotonin, and oxotremorine. In the case of nicotine, lethal bronchoconstriction was produced only in the propranolol-treated animals. The presence or absence of bronchoconstriction was determined by *in vitro* perfusion of the lungs, excised immediately after death.

It has been reported recently that β-adrenergic blockade increases the sensitivity of guinea pigs (1–5) and man (6, 7) to bronchoconstrictors. This potentiation has been attributed to an antagonism of the bronchodilator effect of catecholamines, released as a result of the action of bronchoconstrictors. This suggested that the LD_{50} values of histamine, serotonin, and a cholin-

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omimetic, such as oxotremorine, represent the effect of doses that surmount the antagonistic effect of released catecholamines on the bronchioles. It may explain the negligible effect of injected catecholamines on the toxicity of histamine in guinea pigs which had been observed in preliminary experiments.

In order to determine the degree of potentiation of the action of the above bronchoconstrictors, experiments were performed on guinea pigs. The toxicity of nicotine was also determined on the assumption that in the presence of propranolol this alkaloid may produce death by bronchoconstriction. It is generally accepted that in untreated animals, the lethal effect of nicotine is due to paralysis of the respiratory muscles (myoneural junction blockage) (8). On the other hand, guinea pig bronchiolar chains (9) and perfused bronchioles (10), are strongly contracted by nicotine. Since nicotine is known to be a releaser of catecholamines from the adrenal gland (11, 12), it was assumed that nicotine bronchoconstriction might become lethal, if the β-agonist effects of endogenous catecholamines were blocked by propranolol.

METHODS

Potentiation of the Lethal Effect of Bronchoconstrictors—Using a special technique for intravenous injection in the ear veins of guinea pigs (13), the LD_{50} of the bronchoconstrictors was determined in control and pretreated albino animals, weighing 225–300 g. In each toxicity determination, three doses, graded at 0.3-log intervals were used. Each dose was tested on 15 guinea pigs. Pretreatment consisted of a dose of 10 mg./kg. of propranolol injected intravenously, approximately 15 min. before the dose of the bronchoconstrictor. Only in the case of nicotine, this drug was injected simultaneously with propranolol.

Lung Perfusion—In order to obtain an indication as to the cause of death, lung perfusion experiments were carried out. In control experiments, the guinea pigs were stunned or killed with a head blow, the chest was opened, and the lungs were excised; one lung was tied off and the other was attached through the trachea to a perfusion apparatus (14) containing Krebs-Henseleit solution at 34–36°. The manometer in the apparatus received approximately 20 ml./min. of perfusion fluid. With no outlet, the fluid level increased in the manometer at an approximate rate of 1.5–1.7 ml./sec. In the case of control animals, an average pressure of 20 ml. was sufficient to force the fluid through the bronchiolar tree, and the lung became rapidly distended. When this happened, the perfusion pressure declined until the lung was maximally distended. The highest perfusion pressure (hereafter called critical perfusion pressure) before the lung was filled with fluid was read off the manometer. This is the value recorded in Table I and could be defined as the minimal perfusion pressure required to force the fluid through the bronchioles. In the experiments where lethal doses of bronchoconstrictor were injected, the lungs were excised immediately after the animal died following the injection. The survival time after lethal doses of serotonin, histamine, and nicotine varied from 3 to 5 min. After oxotremorine the survival time was 7–12 min. Those guinea pigs which received the smaller, nonlethal doses of the bronchoconstrictors, (Table II) were killed approximately 5 min. after injection.

The animals injected with propranolol alone were killed 15 min. after injection. In all cases, the lungs were set up for perfusion as described above for the controls.

The drugs tested were histamine diphosphate, serotonin creatinine sulfate, oxotremorine, nicotine D-bitartrate dihydrate, propranolol hydrochloride, and DCI (dichloroisoproterenol HCl). The doses are expressed in terms of the bases.

RESULTS

The toxicity of the bronchoconstrictor in control and propranolol-pretreated guinea pigs is shown in Table II. Propranolol produced a 7- to 26-fold increase in the toxicity of nicotine, histamine, and oxotremorine. The effect on the toxicity of serotonin was considerably more pronounced; the LD_{50} of serotonin in the control was 105 times larger than in the propranolol-treated animals.

TABLE I—GUINEA PIG LUNG PERFUSION

Bronchoconstrictors	Dose, mg./kg. i.v., as Base	β-Blocker	No. of Lungs Perfused	Mean	Critical Perfusion CPP, cm. H_2O; CPP Reached Max. Value 82 cm. H_2O, Number of Lungs
Controls	—	—	12	20	—
—	—	Propranolol, 10 mg./kg. i.v.	6	52.3	—
Serotonin	54	—	11	>70	(6)
Serotonin	0.4	—	6	20.2	—
Serotonin	0.4	Propranolol[a]	7	>79	(6)
Histamine	0.6	—	13	>77	(11)
Histamine	0.03	—	6	22.8	—
Histamine	0.03	Propranolol[a]	5	>79	(4)
Oxotremorine	1.2	—	6	>60	(1)
Oxotremorine	0.012	—	4	27.5	—
Oxotremorine	0.012	Propranolol[a]	5	>80	(4)
Nicotine	3.0	—	5	23.6	—
Nicotine	1.5	Propranolol[b]	6	>79	(5)

[a] Propranolol HCl, 10 mg./kg. injected i.v. 15 min. before bronchoconstrictor. [b] Propranolol HCl, 10 mg./kg. injected simultaneously with nicotine.

TABLE II—EFFECT OF PROPRANOLOL ON THE TOXICITY OF HISTAMINE OXOTREMORINE, SEROTONIN, AND NICOTINE IN GUINEA PIGS

Bronchoconstrictor, i.v. admin.	Propranolol: Dose, mg./kg.	Propranolol: No. of Doses	Propranolol: Route of Admin.	Propranolol: Interval Between Propanolol and Injections	LD_{50} of Bronchoconstrictor mcg./kg., as Base	Toxicity Increase LD_{50} Ratios
Histamine	0	3	—	—	220 ± 19	22
Histamine	10	3	i.v.	15 min.	9.8 ± 1.2	
Serotonin	0	3	—	—	12,800 ± 290	105
Serotonin	10	3	i.v.	15 min.	122.0 ± 15.6	
Oxotremorine	0	3	—	—	162.0 ± 17.0	26
Oxotremorine	10	3	i.v.	15 min.	6.2 ± 0.4	
Nicotine	0	3	—	—	1,080 ± 150	7
Nicotine	10	3	i.v.	Simultaneous	150 ± 38	

In all cases, with the exception of the lethal effect of nicotine in the animals not pretreated with propranolol, death appears to be due to respiratory obstruction; the pressure required to initiate the perfusion of the lungs (critical perfusion pressure) was much greater than in the unmedicated animal dying from a head blow.

Dichloroisoproterenol (DCI) also increased the toxicity of histamine, confirming that the effect is due to a blockade of β-receptors. The i.v. LD_{50} of histamine was reduced from 220 ± 19 to 54 ± 8.8 mcg./kg. 30 min. after the subcutaneous injection of 10 mg./kg. of DCI.

Perfusion Experiments—The results of the perfusion experiments clearly indicate that lethal bronchoconstriction persists after death, as measured by the increased resistance of the bronchiolar tree to perfusion.

The height of the critical perfusion pressure was much larger in animals dying after lethal doses of known bronchoconstrictors (histamine, serotonin, oxotremorine). The smaller doses (histamine: 0.03 mg./kg., serotonin: 0.4 mg./kg., oxotremorine: 0.012 mg./kg.) which were well-tolerated in the nonpretreated guinea pig, did not result in postmortem bronchoconstriction; the critical perfusion pressure readings after administration of those doses were not much higher than those of the control lungs. On the other hand, the highest critical perfusion pressure readings were obtained with lungs from animals which received the same small doses after propranolol pretreatment, strongly suggesting that bronchoconstriction was the cause of death. After the largest dose of nicotine used (LD_{50} × 3) the critical perfusion pressure was not much larger than that of the controls. A smaller dose injected with propranolol resulted in a pronounced postmortem bronchoconstriction.

DISCUSSION

As suggested by Collier *et al.* (1), Townley *et al.* (2, 3), and others (4, 5), the blockade of β-receptors in the bronchioles would prevent the bronchodialator effect of released epinephrine. The bronchoconstrictors the authors have studied could provoke the release of catecholamines from the adrenal glands by two mechanisms: a direct action on chromaffin cells, and an indirect one provoked by asphyxia and mediated through the splanchnic nerves [Cannon and Hoskins (15), Tournade and Chabrol (16), Houssay and Molinelli (17), Houssay (18), and others]. The output of adrenal catecholamines is increased by histamine [Elliott (19), LaBarre (20), Malmejac, Gross, and Neverre (21), Houssay (18)], serotonin [Poisnier and Douglas (22), Douglas (23)], and muscarinic agents (23, 24). The dose of propranolol was sufficiently large to antagonize the β-receptor effect of released epinephrine.

The experiments suggest that the biogenic substances that produce bronchoconstriction also stimulate the release of endogenous bronchodilators (oxotremorine and nicotine mimic the muscarinic and nicotinic effects of acetylcholine, respectively). That this antagonism is fairly efficient is indicated by the fact that the released catecholamines antagonize many lethal doses of the bronchoconstrictors. In the case of serotonin, the endogenous catecholamines appear to protect against more than 100 LD_{50}'s.

The perfusion experiments indicate that maximal bronchoconstriction is the cause of death in guinea pigs receiving nicotine and propranolol simultaneously. On the other hand, in control animals the intravenous injection of a large dose of nicotine (3 × LD_{50}) kills by a different mechanism.

Propranolol alone, injected i.v. 15 min. before death by a head blow, produced moderate increases in critical perfusion pressure. However, the degree of bronchoconstriction was not sufficiently large to produce visible symptoms of respiratory distress during the intervening 15-min. period.

Another point to be considered is the proportions of receptors occupied by the agonists in the presence and in the absence of propranolol. In the propranolol-treated guinea pigs a dose of 400 mcg./kg. of serotonin killed all the animals injected. It was assumed that the contraction of the bronchioles was maximal. In other words, all or most of the serotonin receptors were occupied. In the absence of propranolol enormous doses of serotonin were needed to produce maximal contraction of the bronchioles. This result could be explained, either by accepting the existence of a very large excess of spare serotonin receptors, or by assuming that endogenous catecholamines, acting on β-receptors, reduce the affinity of serotonin to its specific receptor.

SUMMARY

The intravenous toxicity of histamine, serotonin, oxotremorine, and nicotine in guinea pigs was greatly increased by propranolol (10 mg./kg. i.v.). The LD_{50} values in mg./kg. in control and pre-

treated animals were as follows: histamine, 0.22 ± 0.019 and 0.0098 ± 0.0012; oxotremorine, 0.16 ± 0.017 and 0.0062 ± 0.0004; nicotine, 1.08 ± 0.15 and 0.15 ± 0.038; and serotonin, 12.8 ± 0.29 and 0.12 ± 0.016. The toxicity of histamine was also increased by pretreatment with dichloroisoproterenol.

The resistance of the bronchiolar tree to Krebs-Henseleit perfusion in lungs excised after intravenous injection of lethal doses of bronchoconstrictors was much higher than that of lungs from control animals.

Doses of oxotremorine, serotonin, and histamine which did not change the resistance to perfusion, resulted in pronounced postmortem bronchoconstriction when the animals had received an intravenous dose of 10 mg./kg. of propranolol 15 min. before the bronchoconstrictor. A similar phenomenon was observed when 1.5 mg./kg. of nicotine was injected i.v. with propranolol.

The results indicate that in toxicity tests in control guinea pigs endogenous catecholamines exert a powerful antagonistic action against the bronchoconstrictor effect of histamine, serotonin, and oxotremorine. In the case of these three substances this protective mechanism can be overwhelmed by larger doses; the mechanism of death is bronchoconstriction. Nicotine, which is a bronchoconstrictor in the guinea pig pretreated with propranolol, does not kill untreated guinea pigs by producing bronchoconstriction.

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Keyphrases

Bronchoconstrictors—toxicity
β-Adrenergic blockade effect—bronchoconstrictor toxicity
Critical perfusion pressure—guinea pig lungs

Antimicrobial Activity of Dermatomucosal Agents

By LEO GREENBERG

Dermatomucosal agents act in a local manner on the skin and mucous membranes and are, in general, not regarded as materials which alter the microbial flora of the body. The present study was undertaken to determine whether they are, in fact, microbiologically inert. Nine categories of dermatomucosal agents were established and at least six commonly available products were investigated in each category. Products were evaluated for antimicrobial activity by the "small tube method" and by filter disk zone of inhibition method utilizing 12 organisms representative of the normal aerobic skin flora as substrates. Results indicate a wide range of antimicrobial activity among dermatomucosals with the distinct possibility that such products may, in actual conditions of use, alter normal human skin flora leading either to beneficial or deleterious results.

THERE IS, in modern pharmaceutical terminology, a large group of drugs which can collectively be identified as "dermatomucosal agents." These are compounded and dispensed in a multitude of different ways, but they all possess the property of acting in a local manner on the skin and mucous membranes. Some act purely in a physical or mechanical fashion (*e.g.*, demulcents, protectives) while others have a chemical mode of action (*e.g.*, astringents, anti-

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