BLOCKING OF INHIBITORY ADRENERGIC RECEPTORS BY A DICHLORO ANALOG OF ISOPROTERENOL[1]

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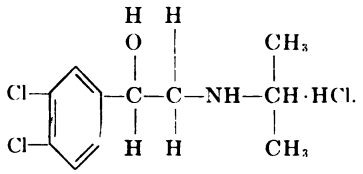
During an investigation of a series of chlorinated phenethyl- and phenethanolamines, it was observed that 1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol hydrochloride selectively blocked some of the inhibitory actions of epinephrine and isoproterenol. Although blockade of the excitatory effects of sympathomimetic amines has often been reported (Nickerson, 1949), only a few examples of antagonism of the inhibitory effects have been published. In 1929, Curtis found that ephedrine contracted isolated strips of both cat and guinea pig uterus, and that after ephedrine, epinephrine failed to relax these organs. The following year Finkleman observed that the relaxing action of epinephrine on isolated rabbit intestine could be blocked by ephedrine. Also using isolated rabbit intestine as the test organ, Astrom (1949) studied the antisympathetic action of a series of sixteen phenethyl amines. All of the compounds, except dihydroxyphenyl-substituted members, blocked the inhibitory action of epinephrine. These compounds were, however, pressor amines with varying degrees of potency. In a study on perfused isolated guinea pig lung, Neidle, Gruber and Copeland (1951) also noted that some sympathomimetic amines were capable of blocking the bronchodilator action of epinephrine.

Dichlorophenyl-2-isopropylaminoethanol differs from the previously reported agents by showing relatively weak inhibitory effects and being virtually devoid of excitatory action. The following report described certain pharmacological actions of this analog.

METHODS. The hydrochloride salt of 1-(3′,4′ dichlorophenyl)-2-isopropylaminoethanol, Lilly 20522, is a white crystalline substance readily soluble in water at room temperature. It is in a sense the dichloro analog of isoproterenol (isopropylnorepinephrine). The structural formula is as follows[2]:



[1] Presented in part at the Federation of American Societies for Experimental Biology Chicago, Illinois, 1957.

[2] This compound was synthesized by Dr. J. Mills, Organic Chemistry Division, Eli Lilly and Company.

Blood pressure of twelve cats, anesthetized with 50 mgm./kgm. of chloralose by vein, was recorded by a mercury manometer. In six female cats, uterine motility was recorded with a gravity writing lever attached by a string to the midpoint of one uterine horn. The two ends of the horn were anchored to a glass organ holder. Blood flow was determined with a Shipley-Wilson rotameter in two female dogs anesthetized with sodium phenobarbital (150 mgm./kgm., i.v.). The rotameter was attached between the divided ends of the femoral artery of the right hind leg with the nerve and venous supply remaining intact. Drugs were injected into the femoral artery distal to the rotameter. Isolated frog hearts (Straub) used in this study were prepared in the usual manner. The smooth muscle action of 20522 was determined on 5 strips of isolated intestine from 3 rabbits and 4 strips of isolated uterus from 3 rats. The isolated muscles were suspended in a constant temperature bath containing Tyrode solution maintained at 37.5°C.

Changes of intrapulmonary resistance to air flow were determined in nine decerebrate-pithed dogs by the method of Jackson (1939). With this method, ventilation was achieved by exerting negative pressure on a Wocher plethysmograph which was placed inside the thoracic cage. The air flow through a tracheal cannula was estimated from a tambour connected to a side arm.

Guinea pig tracheal chains were prepared according to the method of Castillo and deBeer (1947). Preparations were suspended in a 100-ml. muscle chamber and connected to a counter-balanced heart lever having a 15-fold magnification. Temperature of the bath was maintained at 37.5°C. Ringer solution containing twice the usual amount of dextrose and aerated with 95 per cent oxygen and 5 per cent carbon dioxide was used as the bathing medium. Pilocarpine was added in a concentration of 1 mgm. per 100 ml. The responses to epinephrine and 20522 were recorded on smoked kymograph paper after the pilocarpine-induced spasm had reached a stable plateau.

RESULTS. In a series of 12 cats anesthetized with chloralose, compound 20522, in doses of 5 to 10 mgm./kgm. intravenously, caused either a transient fall in blood pressure or no observable change. The degree of blood pressure fall seemed to be related to the rate of injection, for after rapid administration there was usually a definite lowering of blood pressure, whereas in other cases slow injection was associated with no change. The effect of various sympathomimetic amines was tested after the blood pressure had returned to a steady level. As shown in figure 1, 5 mgm./kgm. i.v. of compound 20522 blocked only the secondary depressor effect of epinephrine, had no effect on the pressor response to epinephrine or levarterenol (norepinephrine), and decreased considerably the depressor effect of isoproterenol. However, after 10 mgm./kgm. doses there seemed to be enhancement of epinephrine and levarterenol pressor responses and almost complete block of the depressor response to isoproterenol. In other animals the pressor response to carotid occlusion was unchanged after 5 and 10 mgm./kgm. doses of compound 20522. Similarly, contraction of the nictitating membrane induced by either epinephrine or preganglionic stimulation did not change. The depressor response to either histamine or methacholine remained unchanged. As shown in figure 2, a transient fall in blood pressure followed a rapid injection of compound 20522 (5.0 mgm./kgm., i.v.). The normal uterine movements were not altered. However, after recovery to preinjection level, the pressor response to epinephrine was slightly enhanced and the uterine relaxation previously encountered was inhibited in all of the 6 cats tested. A slight reversal of the uterine response to epinephrine was observed in 2 of 6 cats tested. In all experiments the depressor action and the uterine relaxing activity of isoproterenol were blocked

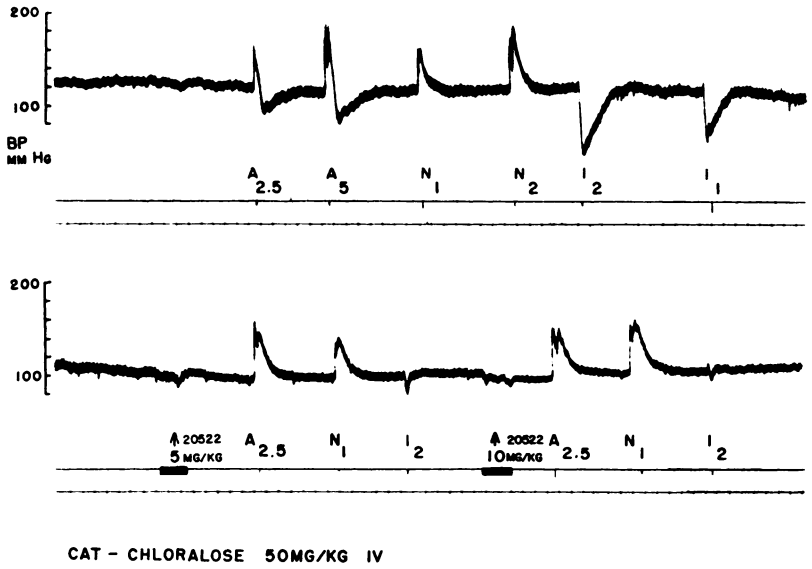


FIG. 1. The blockade of the inhibitory effect of isoproterenol and epinephrine by compound 20522 on the blood pressure of a cat.

Epinephrine (A), levarterenol (norepinephrine) (N) and isoproterenol (I); dose in microgm./kgm.

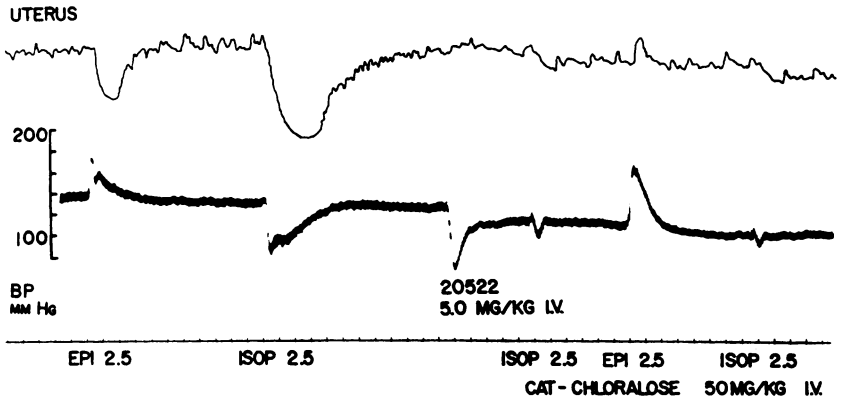


FIG. 2. The blockade of the inhibitory effect of epinephrine (EPI) and isoproterenol (ISOP) by compound 20522 on the uterus *in situ* and blood pressure of a cat.

or substantially reduced. The blocking action of compound 20522 persisted for more than three hours in a few preparations in which the test was prolonged.

Three additional cat blood pressure experiments were done in which the hypertensive effect of epinephrine was blocked by Dibenamine. Ten mgm./kgm. of Dibenamine were given in 5 divided doses, each injected over a period of one minute and followed by a two-minute pause. One-half hour after completion of this series, the injection of 5 microgm./kgm. of epinephrine caused a characteristic fall in blood pressure with little or no preliminary rise. Compound 20522,

5 mgm. kgm., was then given and ten minutes later the depressor response to epinephrine was reduced (see fig. 3). Ten minutes after an additional 10 mgm./kgm. of 20522, the injection of epinephrine caused a pressor response in all three cats, indicating incomplete Dibenamide blockade. One hour after an additional 10 mgm./kgm. of Dibenamine, epinephrine caused a fall in blood pressure less than that first seen. After this, another 10 mgm. kgm. of 20522 was given. Fourteen minutes later, the injection of epinephrine was almost devoid of effect. These experiments confirm the suggestion of Nickerson (1949) that 20 mgm./kgm. of Dibenamine is sufficient to block the excitatory effects of epinephrine and indicate that 20 to 25 mgm. kgm. of 20522 will probably block the inhibitory effects.

In two dogs, injection of isoproterenol into the femoral artery distal to a

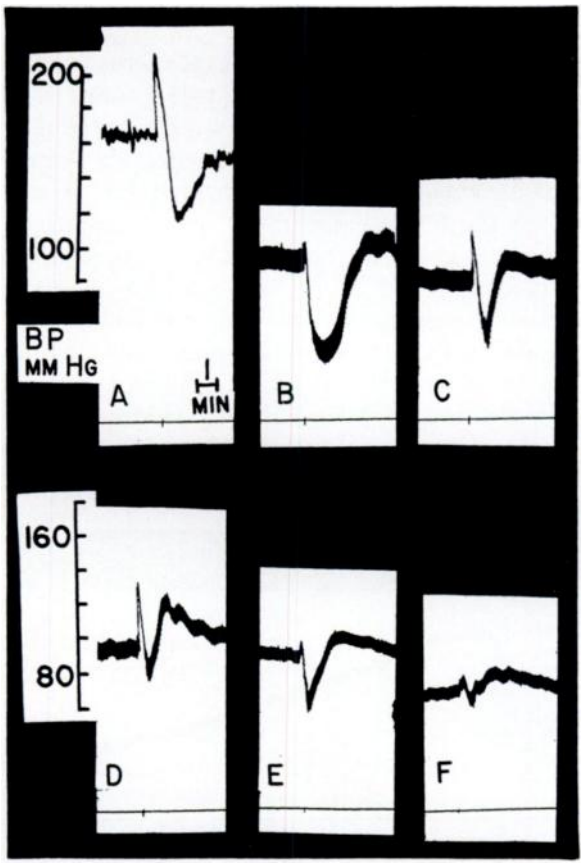


FIG. 3. Selective blockade of the excitatory and inhibitory effects of epinephrine by Dibenamine and 20522.

In each block, epinephrine 5 microgm./kgm. was given. A—before either blocking agent. B—30 minutes after Dibenamine, 10 mgm./kgm. C—20 minutes after 20522, 5 mgm./kgm. D—18 minutes after an additional 10 mgm./kgm. of 20522. E—52 minutes after an additional 10 mgm./kgm. of Dibenamine. F—14 minutes after an additional 10 mgm./kgm. of 20522.

Shipley-Wilson rotameter caused a transient increase in blood flow. A 1 mgm./kgm. dose of compound 20522 given by the same route did not alter blood pressure and pulse rate but there was a transient increase of blood flow through the artery. When the blood flow returned to normal, administration of isoproterenol caused an increase in flow of only 40 per cent of that which had been observed prior to treatment with compound 20522.

In three experiments with the Straub isolated frog heart preparation, administration of compound 20522 in a concentration of 1–100,000 not only failed to alter either the rate or force of contractions of the heart but also failed to change the response to epinephrine. More concentrated solutions decreased both force and rate of the heart beat and at the same time blocked the inotropic and chronotropic effects of epinephrine. Because of the considerable decrease in heart rate and contractility seen after compound 20522, the specificity of this response remains in doubt.

Normal rhythmic movements of the isolated rat uterus in all four preparations were consistently inhibited by isoproterenol with concentrations as dilute as 1–2,000,000. Doses of the analog which did not inhibit uterine motility—10 and 20 microgm. per 100 ml.—failed to alter the effect of isoproterenol. With a dilution of 1–100,000 compound 20522 completely inhibited motility of the rat uterus. However, on the five isolated rabbit intestinal strips, a 1–10,000,000 concentration of the analog did not change activity of the strips but did diminish inhibitory effects of isoproterenol.

When intrapulmonary resistance to air flow was determined in decerebrate-pithed dogs according to the method of Jackson (1939), administration of 0.5 mgm./kgm. of ergotoxine caused a slight bronchoconstriction. In some experiments this slight degree of bronchoconstriction could be partially overcome by 5 and 10 mgm. kgm. doses of compound 20522. In our usual tests for bron-

FIG. 4. Antagonism by 20522 of the bronchodilator action of epinephrine on pilocarpine-induced bronchospasm in decerebrate-pithed dog.

The upper tracing records the airflow through the tracheal cannula and the lower tracing, blood pressure. Epinephrine, 6 microgm./kgm. given at each A. At E, ergotoxine, 0.5 mgm./kgm., and at P, pilocarpine, 0.1 mgm./kgm., were given intravenously. Compound 20522, 3 mgm./kgm., was given at point indicated after it had been demonstrated that epinephrine caused transient bronchodilatation. After the administration of 20522, epinephrine-induced bronchodilatation was reduced.

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