THE REFLEX RESPIRATORY AND CIRCULATORY ACTIONS OF SOME CINCHONINIC ACID DERIVATIVES AND A NUMBER OF UNRELATED COMPOUNDS[1]

HARRY A. WALKER, SUSANNE WILSON, CAROLYN FARRAR AND ARTHUR P. RICHARDSON

Department of Pharmacology, Emory University School of Medicine, Emory University, Georgia

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A wide variety of compounds has been shown (Aviado *et al.*, 1949, 1950; Dawes and Fastier, 1950; Dawes and Mott, 1950; Walker *et al.*, 1951) to produce, on intravenous injection, reflex respiratory and circulatory effects (apnea, fall of blood pressure and bradycardia), which appear to be similar to the Bezold phenomenon (Bezold and Hirt, 1867). Further investigations on the reflex actions of 3-hydroxy-2-phenyl cinchoninic acid (HPC) and related compounds are described in this paper in addition to observations on a number of other chemically unrelated compounds producing similar responses in the dog and cat.

METHODS. The methods employed were essentially those reported previously (Walker *et al.*, 1951). Dogs and cats anesthetized with chloralose and morphine or Dial-urethane were used. All drugs were given intravenously. The activity of the compounds is expressed as the minimum dose producing significant changes in respiration, blood pressure and heart rate as determined from at least three experiments with each compound.

RESULTS. *HPC and Derivatives:* A number of derivatives[2] of HPC previously investigated for their antidiuretic action was studied for their reflex activity in a series of 45 dogs and compared with HPC, which was taken as a standard. The minimal effective dose of HPC for both its antidiuretic and reflex activity was 5 mgm./kgm.

The results of this study together with the chemical structure and the antidiuretic activities as reported by Marshall, Blanchard and Dearborn (1950) and Blanchard *et al.* (1951), are shown in table 1 and represent the activities as ratios of the doses of HPC and the compounds producing equivalent responses. There appears to be no correlation between these two activities, except that the 8-carboxy derivative proved to be the most potent compound in both respects and that a hydroxyl group in the 3-position was essential for both actions. In a few isolated experiments esterification of both carboxyl groups or acetylation of the hydroxyl group of the 8-carboxy derivative of HPC generally decreased the reflex activity by $\frac{1}{2}$ to $\frac{1}{4}$.

Except for 3-hydroxy cinchoninic acid (HCA), all HPC derivatives which exhibited reflex activity also demonstrated the phenomenon of tachyphylaxis

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[2] Generously supplied by Dr. E. K. Marshall, Jr., Johns Hopkins University School of Medicine.

previously reported for HPC (Walker *et al.*, 1951), in that animals were almost completely refractory not only to a second dose of the drug, but also to HPC or any other active derivative. With HPC this phenomenon was shown to be an apparent blockade of certain sensory receptors. However, the previous injection of an effective dose of HCA did not markedly influence the action of a second dose of HCA or a subsequent dose of HPC. On the other hand, the previous injection of an effective dose of HPC did prevent the characteristic reflex response of HCA. This indicates that HCA, unlike HPC, did not effectively block the sensory receptors involved in this response, though the same receptors were initially sensitive to both compounds at the same dosage. A partial explanation for this effect may be made from some unpublished observations of Marshall and Dearborn (1951), which indicated that the plasma levels of HCA fell off rapidly from a single intravenous dose in contrast to HPC.

TABLE 1

Relative reflex and antidiuretic activity of cinchoninic acid derivatives in the dog

SUBSTITUENTS	REFLEX ACTIVITY	ANTIDIURETIC* ACTIVITY
3-hydroxy-2-phenyl- (HPC)	1†	1†
3-hydroxy-2-phenyl-7-chloro-	0.5	5–10
3-hydroxy-2-phenyl-8-carboxy-	4	160
3-hydroxy-2-methyl-	2	0.5
3-hydroxy-2-methyl-7-chloro-	1	5
3-hydroxy-2-methyl-8-carboxy-	1	80
3-hydroxy- (HCA)	0.5	1
2-phenyl-	0‡	0‡

* Data of Marshall, Blanchard and Dearborn (1950); Blanchard, Dearborn and Marshall (1951).

† Activity is expressed in terms of 3-hydroxy-2-phenyl cinchoninic acid.

‡ Inactive at 20 mgm./kgm.

Additional experiments were conducted with the most potent member of the HPC series, the 8-carboxy derivative. It had been demonstrated previously (Walker *et al.*, 1951) that the duration of tachyphylaxis or refractoriness to a second dose of HPC was at least eight hours for the circulatory effect. However, with this more active derivative the duration of the refractory period was found to be only 1.5 hours with equipotent doses. Therefore, this derivative produced a significantly shorter blockade of the sensory receptors than did HPC. This was probably due to a more rapid metabolism and/or excretion of this compound, since it has been demonstrated (Marshall and Dearborn, 1950; 1951) that HPC persisted in the plasma of dogs in fairly high concentration for eight to ten hours, while the plasma levels of the 8-carboxy derivative decreased much more abruptly.

Cats were used in ten experiments to determine any additional species differences in the reflex actions of HPC, since it was demonstrated earlier (Walker *et al.*, 1951) that HPC did not exhibit significant reflex activity in the rabbit.

It was found that HPC produced its typical reflex effects in cats with doses similar to those used in the dog experiments, as illustrated in fig. 1. Fig. 1 also shows that the phenomenon of tachyphylaxis was absent or of a low degree of magnitude in the cat experiments as contrasted to the results observed in the dog, indicating further significant species differences in the reflex activities of this agent.

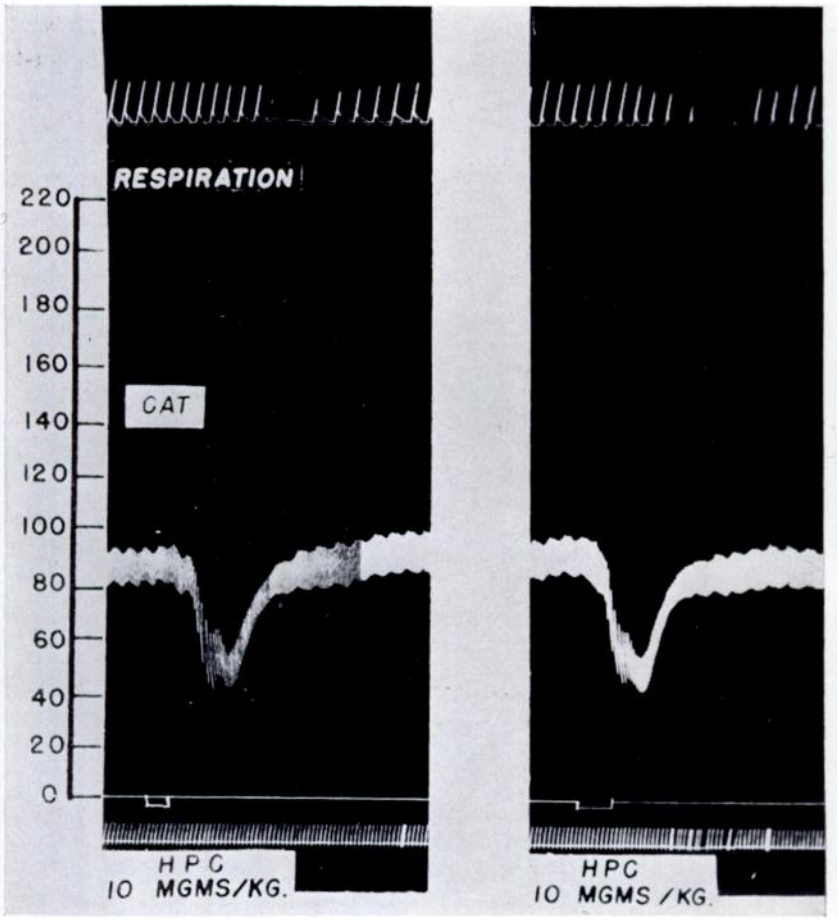


FIG. 1. Effect of successive intravenous doses of 3-hydroxy-2-phenyl cinchoninic acid (HPC) on the respiration, blood pressure and heart rate of the cat. Five minute interval between doses. Time interval is one second.

Phenyldiguanide (PDG) and 2-α-Naphthylethylisothiourea (NETU): This species difference between the dog and the cat in regard to the reflex respiratory and circulatory activity of compounds was particularly demonstrated in the case of PDG and NETU. Dawes and Mott (1950) and Dawes and Fastier (1950) reported the reflex activity of these compounds in cats and rabbits. However, in our experiments with dogs the compounds in doses equal to or up to forty times

greater than those used by the above-mentioned authors did not exhibit a similar activity, but instead showed a definite stimulation of respiration accompanied by a rise in blood pressure. Fig. 2 illustrates the typical responses of PDG in the cat and the dog.

Miscellaneous Compounds: Phenindamine has been shown to possess a Bezold-like reflex activity (Aviado *et al.*, 1950; Walker *et al.*, 1951). Two compounds closely related chemically to phenindamine were also studied in the dog and cat for their reflex activity: 2-methyl-9-phenyl-2,3-dihydro-1-pyridindene (Nu-1326)[3] and 2- methyl-9-phenyl-2,3,4,4a,9,9a-hexahydro-1-pyridindene (Nu-1525).[3] The minimum effective doses of phenindamine, Nu-1326 and Nu-1525

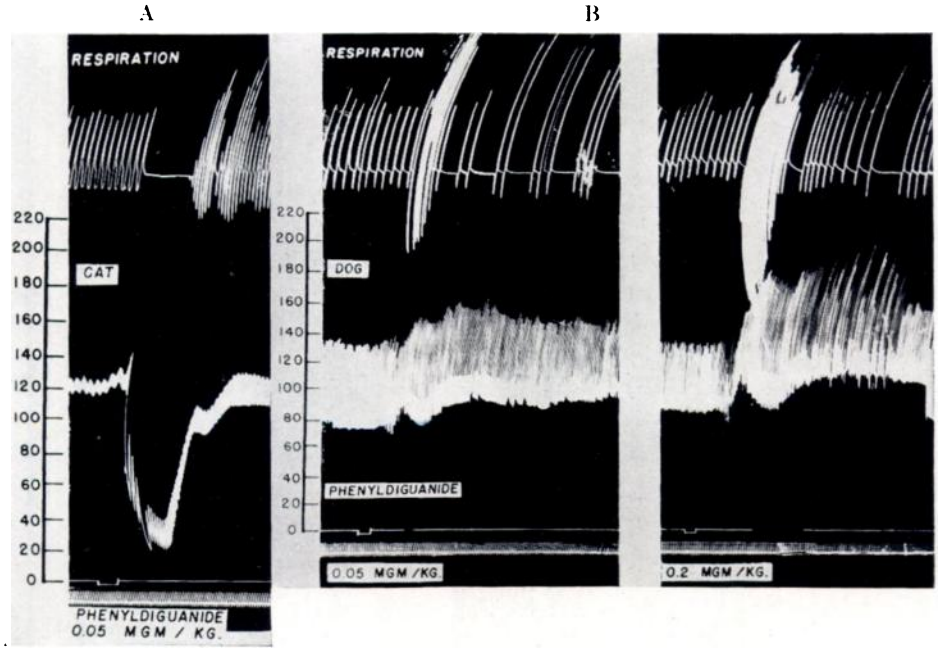
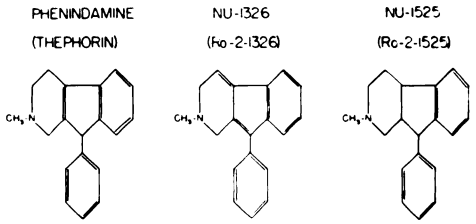


FIG. 2. Effect of intravenous doses of phenyldiguanide (PDG) on the respiration, blood pressure and heart rate of the cat (A) and the dog (B). Lower record is that of blood pressure. Time interval is one second.

were found to be 1, 5 and 0.25 mgm. /kgm., respectively. A comparison of the reflex and antihistamine activities (Lehmann, 1948) of phenindamine and its two derivatives together with their chemical structures is shown in fig. 3. The data confirmed the observation of Aviado *et al.* (1950) that the ability of antihistamine agents to cause reflex apnea was not related to their antihistamine activity, since the most potent in reflex activity, Nu-1525, possessed the least antihistamine activity. In the cat these compounds produced similar reflex effects with equivalent doses (fig. 4). None of these compounds exhibited significant tachyphylaxis in the dog or cat.

[3] Generously supplied by Dr. L. A. Pirk, Hoffmann-La Roche, Inc.



BEZOLD ACTIVITY	1	02	4
ANTI-HISTAMINE ACTIVITY[1]	1	02	003

[1] CATZ OF LEHMANN - J PHARM 82:249/1944

FIG. 3. Comparative reflex and antihistamine activity of phenindamine and two derivatives in relationship to chemical structure.

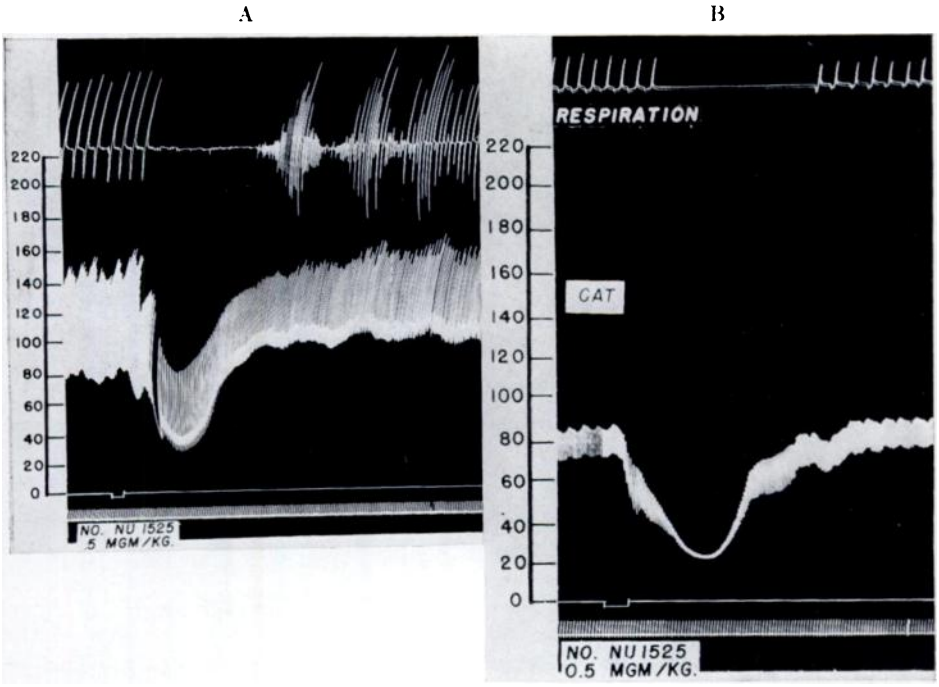


FIG. 4. Reflex respiratory and circulatory effects of intravenous doses of Nu-1525 in the dog (A) and the cat (B). Upper record is that of respiration; lower, blood pressure. Time interval is one second.

Still another compound, 1-diethylamino-3-o-tolyloxy-2-propanol (✱122),[4] was found to produce these same reflex responses in dogs in doses essentially the

[4] Generously supplied by Mr. W. A. Lott, E. R. Squibb & Sons.

same as those of HPC (fig. 5). However, the 1-dimethylamino derivative, a compound more closely related to the antihistamine agents than # 122, proved to be only $\frac{1}{4}$–$\frac{1}{8}$ as active.

Discussion. The data presented herein and elsewhere indicate the presence of certain sensory receptors in the circulatory system of the dog, cat and rabbit

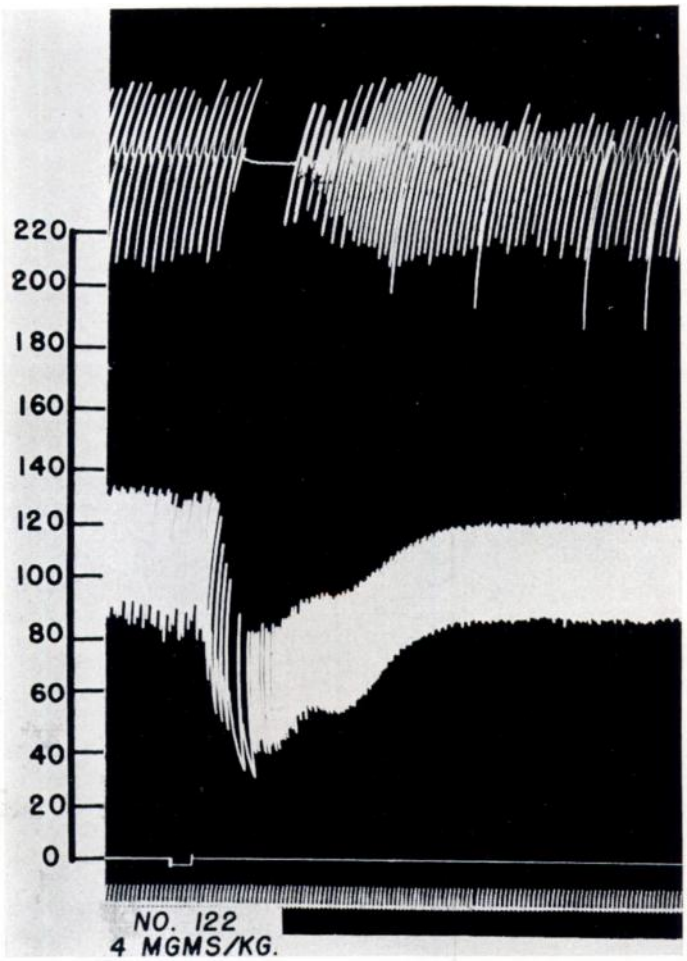


Fig. 5. Reflex respiratory and circulatory effects of intravenous dose of 1-diethylamino-3-o-tolyloxy-2-propanol in the dog. Upper record is that of respiration; lower, blood pressure. Time interval is one second.

which are sensitive to a wide variety of chemical agents, resulting in a unique response of apnea, fall of blood pressure and bradycardia. The sensitivity of these receptors to various compounds differed widely in the same species in that the minimal doses required to produce the typical response ranged from 0.001–0.002 mgm. kgm. of germitrine and veratridine (veratrum alkaloids) to 5–10

mgm./kgm. of the cinchoninic acid derivatives. In addition, a significant species difference has been demonstrated with a number of these agents in the dog, cat and rabbit. The outstanding example was in the case of phenyldiguanide and 2-α-naphthylethylisothiourea, which were very active in the cat (and rabbit) but showed no reflex activity in the dog.

In the dog these receptors were initially sensitive to HPC and several derivatives but were immediately blocked and remained refractory to subsequent doses of the agent for periods of time varying from 1.5 to 8 hours, a fact which could be correlated to some extent with the fate of the compound in the body. However, dogs shown to be refractory to HPC or an active derivative responded typically to the veratrum alkaloids and other chemically unrelated compounds with the production of characteristic reflex respiratory and circulatory effects. From these observations one might conclude that these agents are acting through different receptors or that they are producing their action on the same receptors through different mechanisms.

It has been demonstrated that many of the antihistamine agents exhibit similar reflex effects when injected intravenously. In most of these compounds the dimethylamino group is an integral part of the molecule. In a study of diphenhydramine derivatives, Aviado *et al.* (1950) showed that the substitution of the diethylamino for the dimethylamino group did not alter the ability of the compound to produce reflex apnea. On the other hand, our observations with the compound, 1-diethylamino-3-o-tolyloxy-2-propanol, showed that the introduction of the 1-dimethylamino group markedly decreased reflex activity. It may be concluded from the various inconsistencies in structure-activity relationship as well as the wide diversity in chemical structure and in minimal effective doses of the many compounds exhibiting reflex respiratory and circulatory effects that a common specific chemical action between compound and receptor is apparently not involved in these responses.

SUMMARY

1. 3-Hydroxy-2-phenyl cinchoninic acid (HPC) and a number of derivatives produce Bezold-like reflex effects (apnea, fall of blood pressure and bradycardia) when injected intravenously into dogs. A hydroxyl group in the 3-position was found to be essential for this activity. The most potent derivative in the series was 3-hydroxy-2-phenyl-8-carboxy cinchoninic acid.

2. Since no correlation could be demonstrated between the reflex effects and the antidiuretic activity of a series of cinchoninic acid derivatives, it was concluded that the reflex circulatory effect played only a minor role in the antidiuretic action.

3. Except for the 3-hydroxy derivative (HCA), all cinchoninic acid derivatives which possessed reflex activity also exhibited the phenomenon of tachyphylaxis in dogs. Duration of refractory period for HPC and the 8-carboxy derivative was 8 and 1.5 hours, respectively.

4. HPC produced essentially similar reflex effects in the dog and the cat in the same dosage. However, tachyphylaxis was absent in the cat.

5. Other species differences in reflex activity of several compounds were demonstrated. Phenyldiguanide and 2-α-naphthylethylisothiourea possess potent Bezold-like reflex activity in the cat and rabbit but not in the dog.

6. Phenindamine and two closely related derivatives possess varying intensities of reflex activity in the dog and cat but no correlation between antihistamine and reflex activity was observed.

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