Cardiovascular Effects of Reserpine, Yohimbine, and Reserpine–Yohimbine Mixtures on Intact Anesthetized Dog

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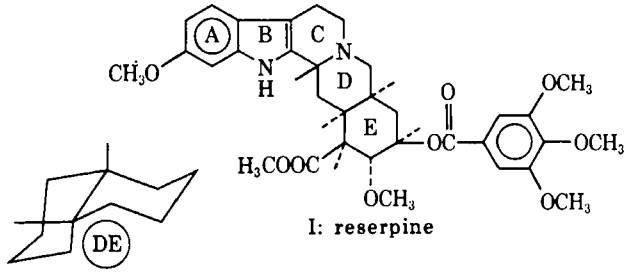
Abstract □ Cardiovascular and respiratory parameters plus responses to periodically injected epinephrine were monitored for 5 hr. in the anesthetized dog after single intravenous injections of saline, reserpine, yohimbine, or reserpine–yohimbine mixtures. Whole root *Rauwolfia serpentina* contains reserpine-like and yohimbine-like alkaloids and is widely used and classified as a reserpine-like agent. However, qualitative results in the present study indicate interactions between reserpine and yohimbine when administered concurrently, with end results incorporating the time–response profiles of both agents when given singly.

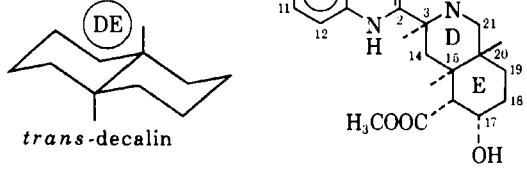
Keyphrases □ Reserpine, yohimbine, and reserpine–yohimbine mixtures—cardiovascular effects on intact anesthetized dog □ Yohimbine, reserpine, and yohimbine–reserpine mixtures—cardiovascular effects on intact anesthetized dog □ Cardiovascular effects—reserpine, yohimbine, and reserpine–yohimbine mixtures, dogs □ Rauwolfia whole root preparations—cardiovascular effects of reserpine, yohimbine, and reserpine–yohimbine mixtures, dogs

Reserpine (I) and yohimbine (II) are naturally occurring pentacyclic tertiary alkaloids whose basic ring systems present a number of similarities. In the former, the D and E rings have a *cis*-decalin conformation; in the latter compound, these rings are *trans*-decalin. Reserpine is esterified with a trimethoxybenzoic moiety on the E ring. The methyl ester grouping at position 16 on the E ring is in a *trans*-position in the reserpine compound, while it is in a *cis*-position in yohimbine. Both compounds possess the α-configuration at the C-15 position. Since the proton at this site does not appear to exhibit a facile epimerization, it may well be that some pharmacological properties of these alkaloids may be due partly to this configuration. Cursory examination indicates that "flipping" with respect to the D and E rings could exist in reserpine, but this would not be the case for the yohimbine molecule. This may also contribute to the different pharmacological profiles of these compounds.



cis-decalin

I: reserpine



trans-decalin

II: yohimbine

Both compounds occur together in many rauwolfia species of the family Apocynaceae. The isolation of reserpine from *Rauwolfia serpentina* Benth may be considered a landmark in the development of modern psychopharmaceuticals. Its isolation also spurred productive investigations in antihypertensive therapy and provoked a general revival of interest in plants as a source of useful and potent therapeutic agents. Our interest in studying selected cardiovascular effects of reserpine, yohimbine, and mixtures of these alkaloids in the intact animal stemmed from the fact that they are concurrently administered therapeutically in medicinals prepared from, or containing, whole root *R. serpentina*. Such whole root preparations antagonize the pressor response to epinephrine (1).

Yohimbine is a classic α-adrenergic blocking agent. It blocks the pressor response to epinephrine and, in sufficient doses, induces "epinephrine reversal" (2). Reserpine is presumed to have no effect on adrenergic receptors, but it depletes central and peripheral stores of catecholamines. This depletion has been reported to hypersensitize adrenergic receptors and potentiate the effect of exogenously administered norepinephrine (3, 4). Yohimbine does not deplete either serotonin or epinephrine centrally (5), but there is evidence that it reduces or depletes norepinephrine in the brain (5). It has also been reported (6) to be an antimetabolite of serotonin. Malone and Roth (7) reported that yohimbine potentiates reserpine-induced blepharoptosis in mice without altering the slope of the dose–response curve. Blepharoptosis is a manifestation of hypothalamic depression and is seen in Horner's syndrome in man (8).

A number of clinical and animal studies demonstrated that preparations made from the whole root of rau-

Table I—Experimental Treatments and 5-hr. Lethality

Treatment at Zero Hour	Dose[a], mg./kg. i.v.	Mean Weight, kg.	Quantal Survival (>300 min.)	Mean Time of Death, min.
0.9% saline	—	14.0	4/4	—
Reserpine	1.0	12.4	4/4	—
Yohimbine	0.1	18.9	4/4	—
Yohimbine	1.0	16.2	4/4	—
Reserpine + yohimbine	1 + 0.1	20.0	3/4	195 (1)
Reserpine + yohimbine	1 + 0.25	14.9	3/4	165 (1)
Reserpine + yohimbine	1 + 1.0	14.1	0/4	26 (4)
Reserpine + yohimbine	0.5 + 0.5	16.6	1/4	129 (3)

[a] Constant dosage of 5 ml. was used.

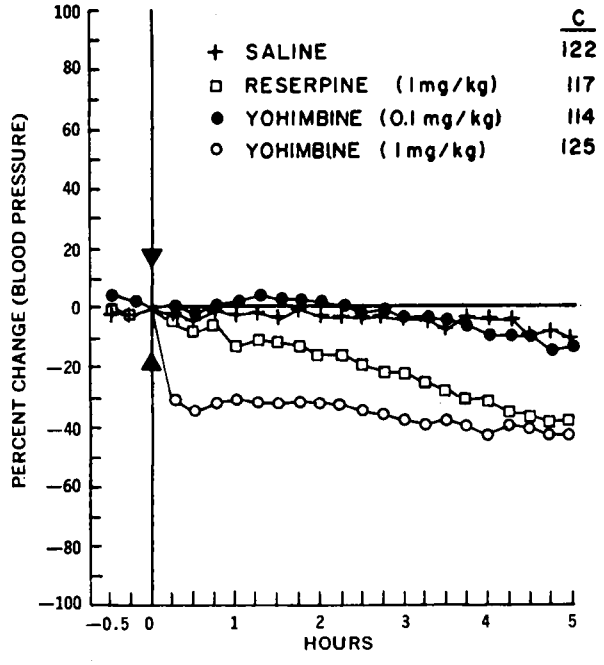


Figure 1—*Blood pressure changes after intravenous administration of isotonic saline, reserpine, or yohimbine expressed as percent change from control (C) values (mm. Hg).*

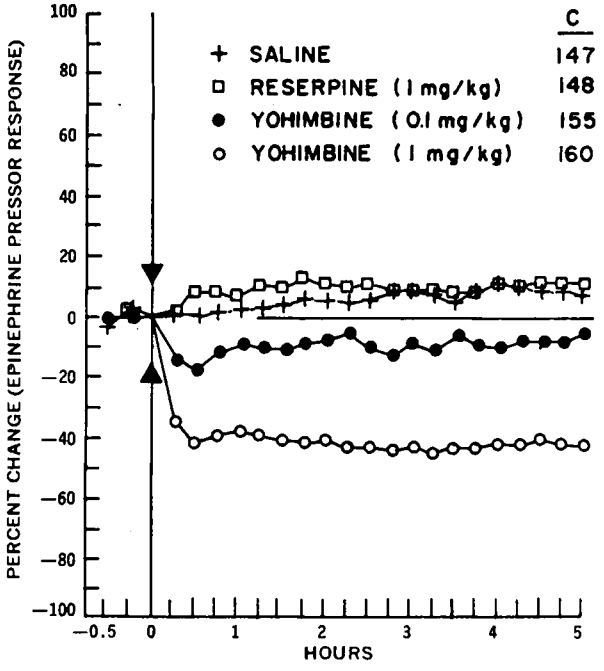


Figure 3—*Changes in epinephrine pressor response after intravenous administration of isotonic saline, reserpine, or yohimbine expressed as percent change from control (C) values (mm. Hg).*

wolfia exhibit greater sedative–hypotensive potency than would be expected from reserpine alone or simply the additive effects of a combination of the various reserpine-like alkaloids (9–15). To date, no experimental study has investigated the cardiovascular dynamics of reserpine–yohimbine mixtures in the intact animal. The reserpine content of *R. serpentina* Benth, collected in the Himalayan foothills, was documented at 0.14% (16). The yohimbine content of such roots has not been reported to date. The ratios of reserpine–yohimbine tested in this cardiovascular study varied from 1.0:1.0 to 1.0:0.1 based on the assumption that the actual content of yohimbine in rauwolfia whole root neither exceeds that of reserpine nor is less than one-tenth that of reserpine.

MATERIALS AND METHODS

Thirty-two mongrel dogs of both sexes, weighing 9.5–40.0 kg., were randomly divided into eight experimental groups (Table I).

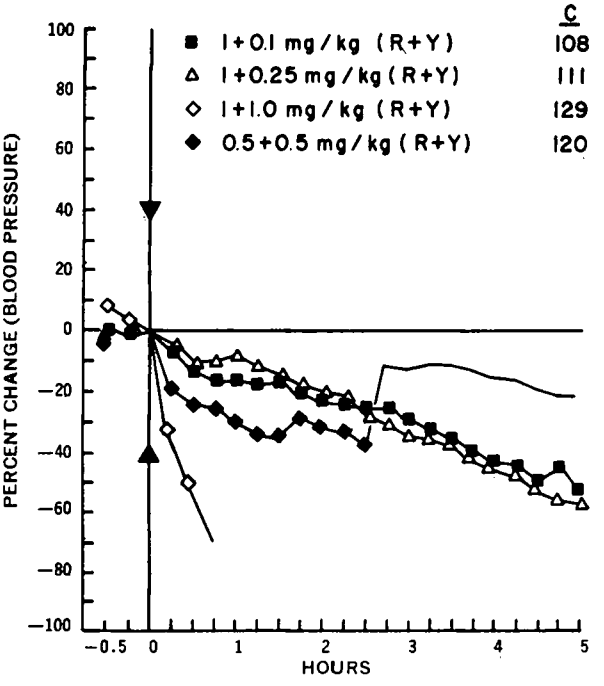


Figure 2—*Blood pressure changes after intravenous administration of nonhypotensive doses of reserpine and yohimbine (R + Y) mixtures expressed as percent change from control (C) values (mm. Hg).*

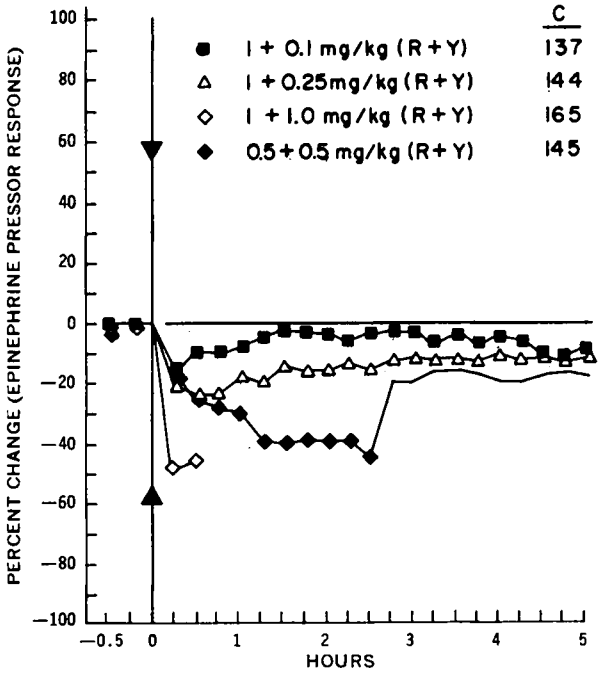


Figure 4—*Changes in epinephrine pressor response after intravenous administration of reserpine and yohimbine (R + Y) mixtures expressed as percent change from control (C) values (mm. Hg).*

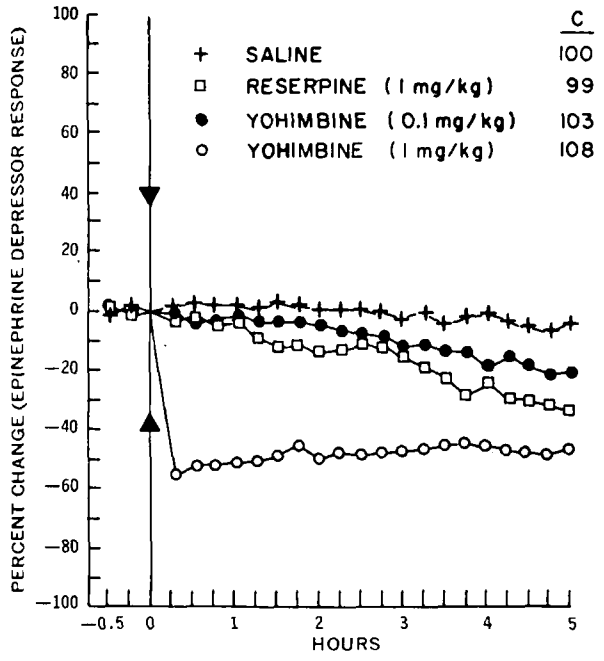


Figure 5—*Changes in epinephrine depressor response after intravenous administration of isotonic saline, reserpine, or yohimbine expressed as percent change from control (C) values (mm. Hg).*

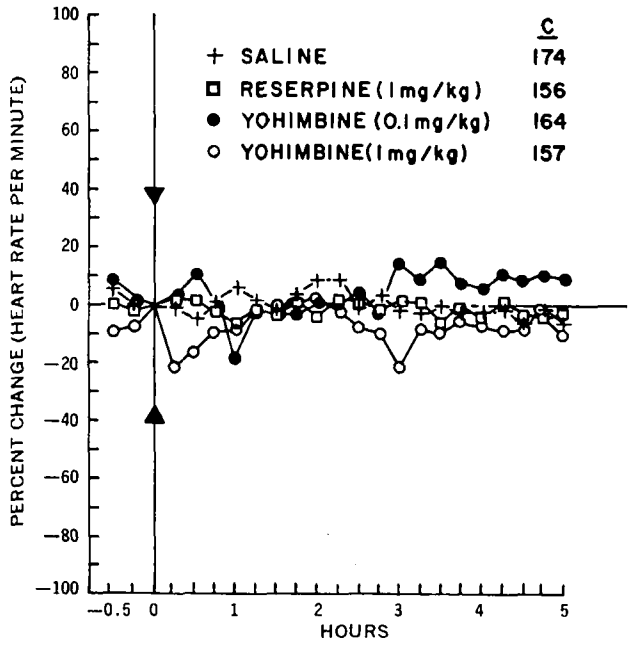


Figure 7—*Heart rate changes after intravenous administration of isotonic saline, reserpine, or yohimbine expressed as percent change from control (C) values (mm. Hg).*

Test animals were taken off feed 12 hr. prior to the start of the experiment with water allowed *ad libitum*. Each animal was anesthetized to the point of blockade of the corneal reflex by slow intravenous titration of a 30% solution of urethan, at a rate of approximately 1 ml./min., *via* the cephalic vein in the left foreleg. Direct femoral arterial blood pressure, heart rate, and respiratory rate were recorded kymographically. After a stabilization period of 15–30 min., a standard dose of epinephrine was determined, intravenously, for each animal. The standard injection (30-sec. duration, with saline wash) was defined as one that would be reproducible at 15-min. intervals and would show a clear biphasic response of pressor–depressor activity. After two control injections of the standard dose of epinephrine, one of the eight treatments was administered at a uniform rate *via* the femoral vein at "zero time." Each injection was of 5-min. duration, followed by a 3-ml. wash of isotonic saline, over 3 min. The total injection time was 8 min.

Reserpine and yohimbine combinations were not administered as a single solution. Reserpine acetate was injected first and washed in with saline. The yohimbine hydrochloride was then injected, followed by a saline wash. The total injection time, for this procedure, also totaled 8 min. Recording of blood pressure, other physiological parameters, and the response to periodic injections of epinephrine was continued throughout the 300-min. observation period unless the test animal died. The control dose of reserpine used (1 mg./kg.) has been shown in this laboratory to produce unequivocal and characteristic effects without lethality.

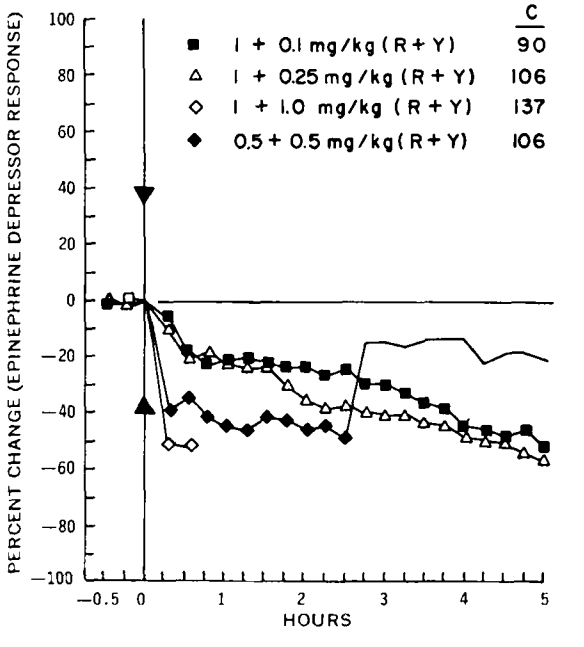


Figure 6—*Changes in epinephrine depressor response after intravenous administration of reserpine and yohimbine (R + Y) mixtures expressed as percent change from control (C) values (mm. Hg).*

RESULTS AND DISCUSSION

Figures 1 through 11 summarize the percent change from control values (preinjection levels of the parameters measured) for each set of treatments. Figure 1 clearly shows the percent change in blood pressure seen after the administration of 1.0 mg./kg. of reserpine as compared to isotonic saline. This dose elicited a slowly developing, progressive hypotension which amounted to a 37% fall at the end of the 5-hr. observation period. This may be contrasted with the absence of such an effect in the salinized animals or those receiving 0.1 mg./kg. of yohimbine. A dose of 1.0 mg./kg. of yohimbine, however, elicited a prompt 32% fall in mean blood pressure. This hypotensive response was maintained throughout the 5-hr. observation period, reaching a maximum of 43% of the control values at the end of the 5th hr.

Concurrent administration of nonhypotensive doses of yohimbine combined with reserpine accentuates the hypotensive effect of reserpine and may cause death. Figure 2 illustrates the effects of such combinations on the percent change in blood pressure as compared to control levels. Injections of 0.1 or 0.25 mg./kg. of yohimbine combined with 1 mg./kg. of reserpine induced essentially equivalent responses, which appeared reserpine-like in onset. However, the effects seen at the end of 5 hr. (54–58%) were considerably lower than the 37% reduction seen with reserpine only (Fig. 1). One animal died during each of these treatments, both in the latter half of the experiment. The combination of 1 mg./kg. each of reserpine and yohimbine was uniformly fatal for two dogs. Both died of respiratory arrest shortly after injection. In the two surviving animals, the resting blood pressure fell to 50% of the control values at the end of 30 min. and respiratory arrest occurred shortly thereafter. Of the four animals treated with 0.5 mg./kg. of each of the two alkaloids, one died after 75 min. and two expired after 150 min.

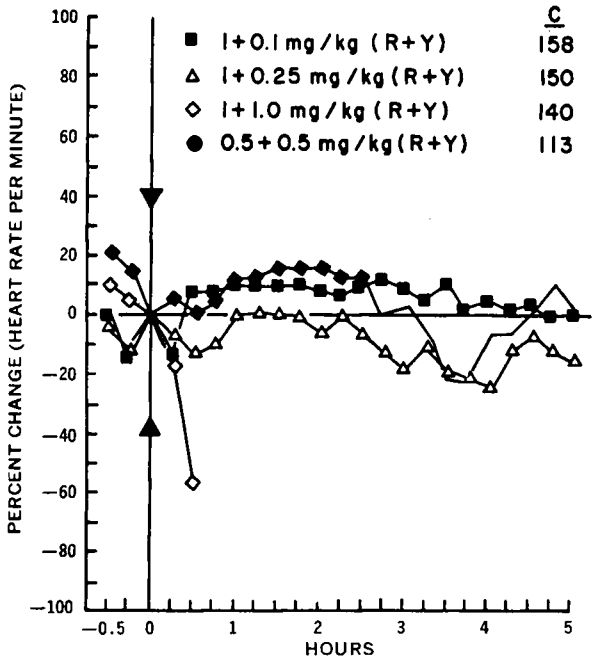


Figure 8—*Heart rate changes after intravenous administration of reserpine and yohimbine (R + Y) mixtures expressed as percent change from control (C) values (mm. Hg).*

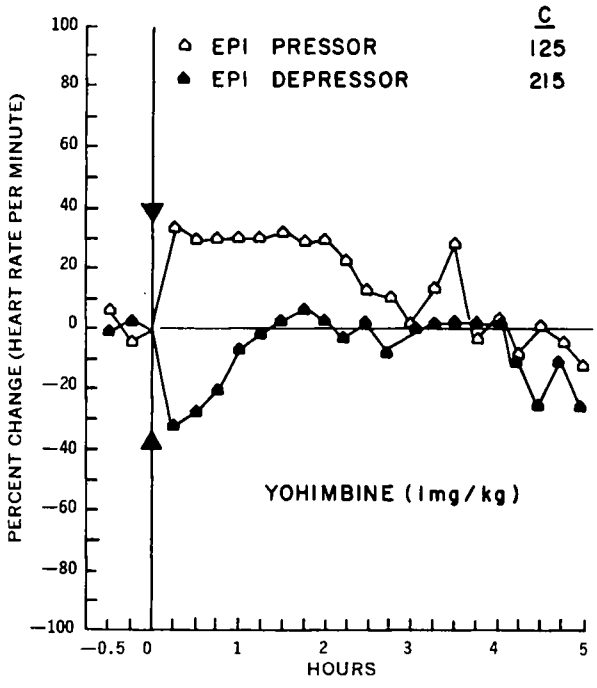


Figure 9—*Changes in epinephrine (EPI) pressor and depressor responses after intravenous administration of yohimbine expressed as percent change from control (C) values (mm. Hg).*

Only one dog survived the full 5-hr. observation period. Its tracing is shown in Fig. 2 as a line without markers. In this test group, the fall in blood pressure was rapid (30% of control levels at 1 hr. and 41% of the controls at the end of 2 hr.).

The effect of the test treatments on exogenous epinephrine is illustrated in Fig. 3. These data indicate that both physiological saline and reserpine enhance the pressor effects of epinephrine about 10% over control values. Bein (17) and Bein *et al.* (18) originally reported a significant enhancement of the epinephrine pressor response after the administration of 0.1 mg./kg. i.v. of reserpine under allobarbital[1] anesthesia. The quantitatively modest results of the present study may be due to species differences or, more probably, the continued repetitive challenges of intravenous epinephrine every 15 min. Such repetitive challenges may prevent the sensitization of adrenergic receptors suggested by Bein. In any event, a dose of 1 mg./kg. of yohimbine has a prompt, unequivocal α-adrenolytic effect which was sustained throughout the 5-hr. experimental period. A dose of 0.1 mg./kg. produced a similar but lesser effect.

Figure 4 illustrates the effects elicited by combinations of reserpine and yohimbine upon the epinephrine pressor response. In all treatments, the effect appears to be what one would expect from yohimbine alone. The effect of the combination of 0.5 mg./kg. reserpine and 0.5 mg./kg. yohimbine is essentially the same as that seen at the lower levels. Even when the lethal combination of 1.0 mg./kg. reserpine and 1.0 mg./kg. yohimbine was used, the α-adrenolytic effect of the combination matched that of yohimbine alone.

Figure 5 illustrates the effect of reserpine on epinephrine's secondary phase. An attenuation of this depressor phase slowly developed over the 5-hr. observation period, at the end of which time a mean value of −34% had been documented. Yohimbine, at a dosage of 1 mg./kg., rapidly attenuated the depressor response by −53% within 15 min. This dramatic effect was sustained in a plateau-like fashion throughout the rest of the observation period. This could be interpreted as β-blocking activity; however, when one considers that the response curves seen here parallel the effects previously presented for resting blood pressure, another conclusion presents itself. From this work and other studies in this laboratory, one can generalize that nonspecific and nonmusculotropic agents which lower the resting blood pressure tend to dampen epinephrine's depressor effect and accentuate the pressor response. Conversely, a nonspecific and nonmusculotropic agent which raises the resting blood pressure tends to accentuate epinephrine's depressor effect and dampen the pressor response.

Figure 6 also shows a direct and passive correlation between the percent decrease in the epinephrine depressor response and the percent decrease in resting blood pressure. Therefore, it was concluded that neither reserpine, yohimbine, nor mixtures of these alkaloids pharmacologically affect the depressor response to exogenous epinephrine.

Figure 7 is concerned with the effects of these alkaloids on the resting heart rate. Treatment with 1 mg./kg. of reserpine did not produce a significant bradycardic effect during the 5-hr. observation

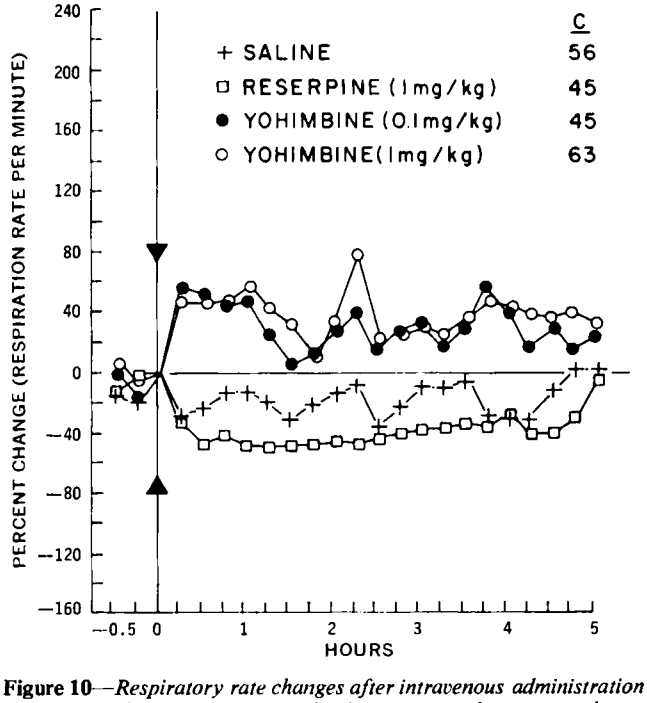


Figure 10—*Respiratory rate changes after intravenous administration of isotonic saline, reserpine, or yohimbine expressed as percent change from control (C) values (mm. Hg).*

[1] Dial.

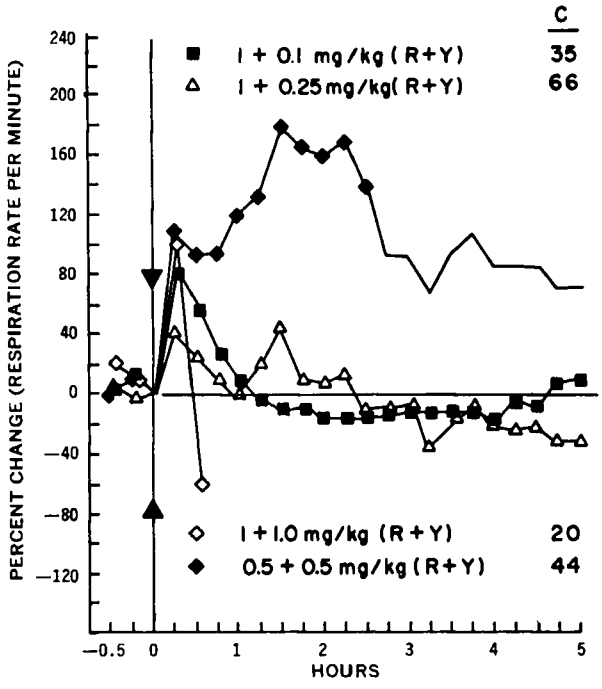


Figure 11—*Respiratory rate changes after intravenous administration of reserpine and yohimbine (R + Y) mixtures expressed as percent change from control (C) values (mm. Hg).*

period. However, 1 mg./kg. of yohimbine produced a prompt 22% slowing of the heart rate, with recovery at the end of the 1st hr. At the 3-hr. interval of the experiment, there was a second period of bradycardia.

Figure 8 exhibits no distinct dose–response pattern. However, a profound and unequivocal bradycardia was seen with the lethal combination of 1.0 mg./kg. of reserpine and 1.0 mg./kg. of yohimbine.

The combination of 1.0 mg./kg. of reserpine and 0.25 mg./kg. of yohimbine elicited a somewhat cyclical bradycardia, which one would not expect considering the effects of reserpine alone (Fig. 7). The effects produced by the combination of 0.5 mg./kg. each of reserpine and yohimbine is shown by the line without markers and is characterized by some equivocal tachycardia seen between 1 and 2 hr. and the bradycardia seen between 3 and 4 hr. Except for the steepness of parts of the first portion of the curve, it bears some similarity to the response seen with the combination of 1.0 mg./kg. reserpine and 0.25 mg./kg. yohimbine.

Figure 9 clearly shows that yohimbine (1 mg./kg.) blocks the vagal slowing seen during the pressor phase of the response to epinephrine. Likewise, it tends to block the tachycardia seen during the depressor phase. This phenomenon was observed with all reserpine–yohimbine combinations and is pharmacologically most interesting. In 1930, Heymans and Bouckaert (19) reported that yohimbine could suppress carotid sinus vasomotor reflexes.

Percentage shifts in respiration were more pronounced than the other parameters measured. Isotonic saline appeared to produce some cyclic depression of the resting respiratory rate, whereas 1 mg./kg. of yohimbine was uniformly depressant (Fig. 10). This depression appeared to be overcome at the end of the 5-hr. observation period. There was some evidence of cyclic stimulation of the respiratory rate in the animals treated with either 1 mg./kg. of reserpine or 0.1 mg./kg. of yohimbine. From Fig. 11, it can be seen that all alkaloid combinations produced a transient stimulation of respiratory rate at the 15-min. interval. Subsequently, the combination of 1 mg./kg. reserpine and 1 mg./kg. yohimbine induced a profound respiratory depression, resulting in the death of all test animals before the end of the 1st hr. of observation. Although it appears in Fig. 11 that treatment with the combination of 0.5 mg./kg. each of reserpine and yohimbine produced only respiratory stimulation, three test animals plunged into respiratory depression during the middle of the observation period and died. Only one dog in this group survived the entire 5-hr. period (its response is noted by the line without markers). After the initial stimulation, the treatment consisting of 1 mg./kg. reserpine and 0.1 mg./kg. yohimbine elicited respiratory responses much like predrug levels. The pattern for the combination of reserpine and yohimbine (1 and 0.25 mg./kg., respectively) showed some evidence of cyclization during most of the observation period.

SUMMARY

It is evident from the data presented that preparations made from the whole root of *R. serpentina* cannot be considered as just other "reserpine-like" drugs. It appears that yohimbine and/or yohimbine-like alkaloids can contribute significantly to the pharmacological–therapeutic profile of rauwolfia whole root.

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