being solely determined by the hypotensive potency of the alseroxylon alkaloids acting by some mechanism other than through the vagi or afferent carotid area nerves.

That the carotid chemoreceptors play no role in the alseroxylon hypotension is indicated by the fact that the blood pressure fell to the same lower levels regardless of whether they were intact or denervated. This fact is verified in the second experiment which is reported elsewhere in THIS JOURNAL.

The transient hypertension seen immediately following the injection of the alseroxylon alkaloids in all of the completely denervated dogs and in a few of the vagotomized and carotid denervated dogs was doubtless due to the acidity and volume of the vehicle, the latter of which varied according to the size of the dog. This was shown by the fact that the appropriate volume of vehicle alone produced exactly the same type of blood pressure curve. This phenomenon was not seen in any of the normal animals because both buffer mechanisms had an over-riding effect; was seen in only certain of the partially denervated dogs because of the variation in potency of the remaining buffer nerves in the different dogs; and was always seen in the completely debuffered animals because of the complete absence of the normal compensatory nervous pathways.

The progressive reduction of the carotid occlusion reflex response following alseroxylon administration has been previously demonstrated and discussed by other investigators (1, 2, 6).

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Rauwolfia Hypotension II.*

Action of the Alseroxylon Alkaloids and Epinephrine on the Carotid Pressoreceptors

By CARL A. SCHLAGEL† and JOHN W. NELSON‡

The action of the alseroxylon alkaloids when injected into the adventitial layer of the carotid bifurcations of vagotomized dogs was investigated. The results showed, as a previous investigation had already indicated, that the Rauwolfia hypotension is primarily mediated through the central nervous system and secondarily through a direct muscular vasodilatory action of these alkaloids. This hypotension is limited in degree and almost devoid of any postural hypotensive aspects because of the incomplete nature of the central blocking action together with the vasodilatory action of the alseroxylon on the carotid sinuses whereby the normal physiological activity of these buffering pressoreceptors is potentiated. A pronounced pressor activity of carotid adventitially-injected epinephrine seen in orally treated Rauwolfia dogs is due to: (*a*) the preexisting hypotension and the Rauwolfia-induced blocking of the central components of the carotid reflex arc; (*b*) the diffusion or otherwise leaking of a portion of the epinephrine dosage into the general circulation; and (*c*) to the action of this systemic epinephrine on the effector end organs which have become sensitized by a Rauwolfia-engendered (pharmacologically-induced) sympathectomy.

IN A PREVIOUS ARTICLE in THIS JOURNAL, it was shown that the hypotension elicited by the alseroxylon alkaloids in dogs was not mediated through either the vagal or carotid nervous pathways. However, these observations did not entirely eliminate these nerves from further consideration as two of the possibly many sites of Rauwolfia action because it was also shown that when these nerves were intact, the alseroxylon hypotension was less intense or somewhat moderated in degree. Whether this buffering action was due solely to the normal function of the sinoaortic receptors reacting to the abnormal reduction in blood pressure, or also to a secondary local action of the alkaloids acting directly on these receptors was not studied in the previous experiment. Accordingly, the present investigation was designed to test the action of the alser-

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† Fellow of the American Foundation for Pharmaceutical Education. Present addres: The Upjohn Company, Kalamazoo, Mich.
‡ Present address: College of Pharmacy, The Ohio State University, Columbus 10.
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oxylon alkaloids when administered locally at the carotid bifurcation. Such a study should add weight to the previous investigation, and should give some clues as to why the Rauwolfia preparations are only mildly acting antihypertensive agents. It should also help explain the usual absence of postural hypotension following Rauwolfia therapy.

EXPERIMENTAL

The animals employed, the surgical techniques used, and the methods for recording blood pressure and carotid occlusion reflex responses have been described elsewhere in THIS JOURNAL. In preliminary trials utilizing 10 dogs, an aqueous extract of *Rauwolfia serpentina*, a commercial solution of epinephrine 1:1000 (Parke-Davis), and a 0.2% solution of $NaNO_2$ in normal saline were used. The Rauwolfia preparation had previously been shown in this laboratory to possess immediate, potent, and prolonged hypotensive activity when injected intravenously in dogs (1). It was used to attempt to verify the suspected Rauwolfia potentiating effect on the pressor-buffering action of the carotid receptor areas and in lieu of the alseroxylon alkaloids and reserpine in order to conserve the limited supply of these latter alkaloids. The epinephrine hydrochloride and $NaNO_2$ were chosen because of their known action on the carotid receptors when injected locally at the carotid bifurcation.

These preliminary trials were also primarily conducted in an effort to determine the final experimental design to be employed in the main body of this study. Accordingly, the blood pressure effects of the above three drugs were compared by applying the drugs to the carotid bifurcations with cotton swabs, by injecting 1.5-ml. volumes of the same drugs into each of the carotid conjunctival spaces, or by injecting 0.1 ml. of the preparations directly into the adventitial layers of the carotid arteries at the region of the bifurcations. In addition, the above three drugs were administered by the above three routes in normal dogs (all nerves intact), in vagotomized dogs, and in vagotomized and unilateral carotid denervated animals. In the latter case, the drugs were administered to only the remaining intact carotid area.

In all instances, the blood pressure changes with the Rauwolfia extract (pressor response) with epinephrine (depressor), and with $NaNO_2$ (pressor) were analogous qualitatively. The quantitative changes, however, were less erratic and usually more potent only following the intra-adventitial injection technique in the vagotomized animals. Unilateral carotid denervation with contralateral drug injection offered no particular advantage. Therefore, in the remainder of this experiment, all the animals were vagotomized in order to limit the reflex regulation of blood pressure to the carotid receptor areas. Likewise, all the drugs, unless otherwise specified, were injected in a total volume of 0.1 ml. into the adventitia of each of the carotid arteries at the region of the bifurcations. The injections were made with a 0.25-ml. syringe using a 27-gauge needle made considerably thinner by filing. The actual technique involved injecting one-half the volume (0.05 ml.) into the adventitia of both the lateral and medial aspects of each carotid receptor area giving a total of four injections for each single administration of drug.

Part I.—Various doses of the alseroxylon alkaloids were injected bilaterally into the carotid areas and their effects on the blood pressure noted. Because these results, as well as the above preliminary observations with the aqueous Rauwolfia extract, showed a pressor action on the carotid receptors which suggested a possible adrenolytic activity, 500-mcg. doses of the alseroxylon alkaloids were injected into the carotid areas both alone, before, and following similarly injected 100-mcg. doses of epinephrine always as the hydrochloride. In a majority of the dozen dogs of this section, 0.1-ml. doses of the citric acid vehicle were injected toward the termination of each experiment. An additional animal received several 0.1-ml. doses of the vehicle alone. Reserpine was studied alone in two animals and together with the alseroxylon alkaloids in another animal for comparison purposes.

Part II.—To study further the pressor response that always occurred following the carotid adventitial injection of appropriate doses of the alseroxylon alkaloids as shown in the results of Part I, four dogs were given 0.5 mg./Kg. of the alseroxylon fraction orally each day for a period of ten days. Two other dogs received 50.0 mcg./Kg./day of reserpine in an identical manner. The tenth dose was given two hours before the administration of the anesthetic on the day the animal was experimented upon. All these animals were then vagotomized and the effects of carotid occlusion and injection of various drugs such as the alseroxylon alkaloids, epinephrine, $NaNO_2$ and vasopressin (Parke-Davis) into the carotid bifurcation were noted. In one of the above orally-treated reserpine animals, a comparison was made between the blood pressure effects of epinephrine injected intravenously and into the carotid area both before and after an appropriate epinephrine reversing dose of Dibenamine®. The effects of carotid area injections of epinephrine and the alseroxylon alkaloids were also studied in one dog which had been given 1.0 mg./Kg. of the alseroxylon fraction six hours previously, and in which the carotid occlusion pressor reflex was blocked to a similar degree as that observed in the orally treated animals.

RESULTS

Part I.—The bilateral injection of doses of 1.0 mcg. of the alseroxylon alkaloids produced no changes in the blood pressure whatsoever. Doses of 10, 25, 50, and 100 mcg. produced only slight pressor responses (5 to 20 mm. Hg), which were transient in nature.

Because of a difference in solubility, only 50 mcg. of reserpine could be dissolved in 0.1 ml. of the citric acid vehicle. When this dose was injected into each sinus region, either no change in blood pressure occurred, or, in a few instances, a slight but transient hypertension was recorded. In another dog, 150 mcg. of reserpine dissolved in 0.3 ml. of vehicle was injected into each sinus region. A 10 mm. Hg rise in pressure occurred after the first dose, while no change was observed after the second, similar dose. The same doses of reserpine and the alseroxylon alkaloids (150 mcg. in 0.3 ml.) were then given at

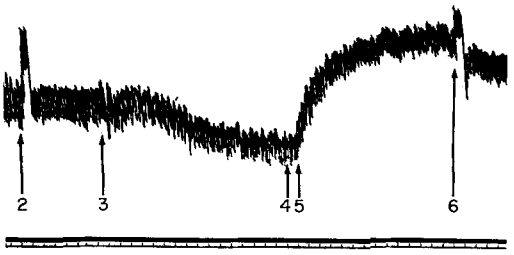

Fig. 1.—Effects of carotid adventitially-injected epinephrine and alseroxylon alkaloids on the blood pressure of a vagotomized dog. Male dog, 16.6 Kg.; lower tracing: time in minutes. *2*, 4:00 carotids clamped, B. P. 148 to 206 mm. Hg; *3*, 4:23, epinephrine, 100 γ into each carotid area, B. P. 144 to 96 mm. Hg; *4*, carotids clamped at 4:39, no change in B. P.; *5*, 4:40, 500 γ of alseroxylon in each carotid area, B. P. 96 to 208 mm. Hg; *6*, effects of carotid clamping restored, B. P. 208 to 228 mm. Hg.

different intervals in still another animal. The reserpine raised the blood pressure only 6.0 mm. Hg, while the alseroxylon alkaloids produced a 20.0 mm. Hg elevation.

Following the bilateral injection of 250 to 500 mcg. of the alseroxylon alkaloids, almost immediate and rather large elevations in the blood pressure occurred. The pressure remained elevated for some time, usually at least one hour, and in several instances, for two hours or longer. During the hypertensive period, the carotid occlusion reflex response was usually not blocked completely but was considerably depressed percentage-wise due to the high blood pressure level previously established by the Rauwolfia alkaloids.

All of the results obtained with the 500-mcg. doses of alseroxylon and 100-mcg. doses of epinephrine were the same qualitatively but varied quantitatively depending upon the exact sequence of dosage employed. For instance, when epinephrine was injected initially, the blood pressure always fell to a level at which the carotid occlusion reflex was blocked. When given during the pressor response to the alseroxylon alkaloids, the ensuing hypotension was very much less impressive, belated, or did not occur at all. On the contrary, when the alseroxylon alkaloids were administered during the nadir of an epinephrine response, the effects of epinephrine were reversed as shown in Fig. 1 (blood pressure elevated to a level higher than the pre-injection norm), or the blood pressure was simply returned to its pre-epinephrine level. In every instance, the alseroxylon alkaloids were able to re-establish the carotid occlusion response which had previously been blocked by action of the epinephrine.

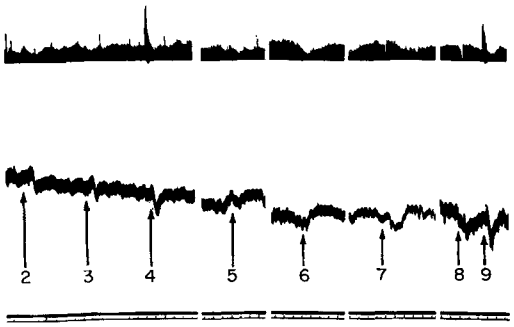

Fig. 2.—Lack of blood pressure response following carotid adventitially-injected epinephrine and alseroxylon alkaloids on a vagotomized dog with the carotid pressoreceptors selectively denervated, leaving the carotid bodies intact. Female dog, 9.3 Kg.; upper tracing: respiration; lower tracing: time in minutes. *2* and *3*, carotids clamped, little change in B. P. indicating pressoreceptors denervated; *4*, NaCN, 1.0 mg./dog i. v., hyperpnea indicating chemoreceptors intact; *5*, alseroxylon, 500 γ in each carotid area, little change in blood pressure; *6*, epinephrine, 100 γ in each carotid area $1^1/_2$ hr. after *5*, little change in blood pressure; *7*, alseroxylon repeated 20 min. after *6*, little change in pressure; *8*, carotids clamped 1 hr. after *7*, little change in pressure; *9*, NaCN, 1.0 mg./dog i. v., hyperpnea indicating chemoreceptors still active and intact.

The carotid adventitial injection of 500 mcg. of alseroxylon in the two dogs which were completely denervated with the exception of having intact chemoreceptors produced no change in the blood pressure as shown in Fig. 2. A total of four such doses was administered to these two animals.

The injection of several doses of the vehicle alone (0.75% citric acid) in the majority of the above animals was without effect. The vehicle was also without effect when given to one animal which had received no previous medication other than the anesthetic. That the carotid sinuses were responsive in this animal was shown by the later injection of the alseroxylon alkaloids and by occlusion of the carotid arteries.

Part II.—The blood pressures and heart rates of the six orally treated and vagotomized animals are shown in Table I. These values were obtained before any drugs were injected into the carotid sinus areas.

TABLE I.—THE MEAN ARTERIAL BLOOD PRESSURES AND HEART RATES OF SIX VAGOTOMIZED DOGS WHICH HAD RECEIVED DAILY ORAL DOSES OF THE RAUWOLFIA ALKALOIDS FOR A PERIOD OF TEN DAYS

Animal	Drug	Dose/day	M. A. P. (mm. Hg)	H. R. (beats/min.)
1	Alseroxylon alkaloids	0.5 mg./Kg.	120	120
4	Alseroxylon alkaloids	0.5 mg./Kg.	90	110
4	Alseroxylon alkaloids	0.5 mg./Kg.	96	104
4	Reserpine	50 mcg./Kg.	93	118
5	Alseroxylon alkaloids	0.5 mg./Kg.	132	98
6	Reserpine	50 mcg./Kg.	131	110

Other results obtained in the four alseroxylon treated and in one of the reserpine animals were as follows: (*a*) an almost complete blocking of the hypertensive response which is reflexly elicited in normal animals by occlusion of the carotid arteries; (*b*) an almost complete blocking of the hypertensive response which is reflexly elicited in normal animals by the carotid adventitial injection of 500 mcg. of the alseroxylon alkaloids; (*c*) a very marked rise in the arterial pressure following

the injection of 100 mcg. of epinephrine into the adventitia of the carotid bifurcations (this is opposed to the depressor response elicited by similarly injected epinephrine in normal animals); similar but not identical responses when 100 mcg. of epinephrine was injected into the carotid bifurcation as when 2–3 mcg. of epinephrine was administered intravenously; (*d*) almost identical elevations in the blood pressure when epinephrine was injected into the carotid area either before or after complete carotid area denervation. A record of one of these animals which depicts most of these responses is shown in Fig. 3.

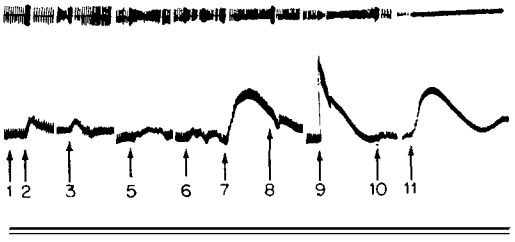

Fig. 3.—Blood pressure responses produced by carotid adventitially-injected alseroxylon and epinephrine in a vagotomized dog following a ten-day oral alseroxylon treatment at a dose of 0.5 mg./Kg./-day. Female dog, 11.0 Kg.; upper tracing: respiration; lower tracing: time in minutes. *1*, normal tracing, B. P. 92 mm. Hg; *2*, *3*, carotids clamped twenty-five minutes apart, carotid reflex response not quite completely blocked; *5*, *6*, alseroxylon, 500 γ into each carotid area twenty-five minutes apart, pressor responses similar to those elicited by carotid clamping; *7*, repeat *4* which is not shown, epinephrine, 100 γ into each carotid bifurcation, marked pressor response rather than depressor as in normal dogs; *8*, carotids clamped during fall in B. P., slight alteration in B. P.; *9*, epinephrine, 50.0 γ/dog i. v., very steep and more potent rise in B. P.; *10*, carotids clamped; *11*, epinephrine repeated as in *7*.

There are some other interesting aspects to this peculiar epinephrine-produced pressor response which were not mentioned in the above account. For instance, the same qualitative blood pressure response, but one of considerably less magnitude, could be obtained by application of epinephrine (1:1000) to the carotid sinus region with a swab. If the carotid sinus areas were denervated with phenol, or injected with 500 mcg. of the alseroxylon alkaloids, or if the carotids were occluded at the onset or during the fall in blood pressure which followed the pressor aspect of the epinephrine response, the blood pressure kept right on falling just as it would have had these experimental techniques not been instituted.

Pressor responses were also recorded following carotid area injections of 0.1 ml. (2 units) of vasopressin or 0.1 ml. of a 1% solution of ephedrine sulfate. However, these responses were of considerably less magnitude than those following epinephrine being on the average 24 mm. Hg for vasopressin and 22 mm. Hg for ephedrine sulfate. The pressor responses to these latter two drugs were also less sharp (delayed), but were progressive and persistent, sometimes for one hour or more. The intra-adventitial injection of 0.1 ml. of 0.2% $NaNO_2$, on the other hand, caused a fall in pressure of approximately 30 mm. Hg. This depressor response was also delayed (5–10 minutes) but persisted for an hour or more. In all instances, the degree of the pressor responses with vasopressin and ephedrine sulfate, and the depressor responses following $NaNO_2$ appeared to be dependent upon the pre-injection blood pressure level.

For comparing the responses in the long term, orally treated to the intravenously treated animals, 3.0 mg./Kg. of the alseroxylon alkaloids were administered intravenously to a vagotomized dog. After six hours, the blood pressure had only receded to 94 mm. Hg and the carotid occlusion was still capable of elevating the pressure 40 mm. or to 134 mm. Hg. The injection of 100 mcg. of epinephrine into the carotid area of this animal raised the blood pressure only from 94 to 100 mm. Hg after five minutes, whereupon it fell to 92 mm. Hg where it remained stationary for at least one-half hour.

Since it was apparent from this animal and from those reported upon in the previous article that 1.0 mg./Kg. given intravenously was capable of producing maximum blood pressure effect, 1.0 mg./Kg. was then given to another vagotomized animal by intravenous injection. After six hours the blood pressure was down to 84 mm. Hg and carotid occlusion was only effective enough to produce a 16-mm. rise.

The intra-adventitial injection of 100 mcg. of epinephrine at this point elevated the blood pressure only 10 mm. Hg. The pressure then receded to 70 mm. Hg which was approximately 12 mm. below the pre-injection level. After one hour, 400 mcg. of the alseroxylon alkaloids elevated the pressure from 70 to 82 mm. Hg or to the pre-epinephrine injection level. At this point, 150 mcg. of epinephrine elevated the pressure to 126 mm. Hg. One-half hour later the pressure was stabilized at 86 mm. Hg. Vasopressin and $NaNO_2$ in this animal produced an elevation and reduction in pressure, respectively, which was analogous to their effects in the orally pretreated animals.

The last animal studied in this experiment was a reserpine pretreated dog. Just as in the other orally treated animals, both intravenous and carotid adventitial injections of epinephrine produced similar pressor responses. However, when identical doses of epinephrine were again administered following an epinephrine reversing dose of Dibenamine, depressor responses were obtained which were almost mirror images of the pre-Dibenamine pressor blood pressure curves. The blood pressure tracing for this animal is shown in Fig. 4.

DISCUSSION

The pressor response which was always obtained following the injection of from 250 to 500 mcg. of the alseroxylon alkaloids into the adventitia of the carotid bifurcations shows that this Rauwolfia fraction has a very definite local action on the nervous receptors of this area. This pressor action is in complete agreement with the results of the previous study reported elsewhere in THIS JOURNAL, where it was shown that the carotid receptors displayed a definite pressor, buffering capacity following the intravenously administered alseroxylon alkaloids. Both of these studies likewise limit the site of this

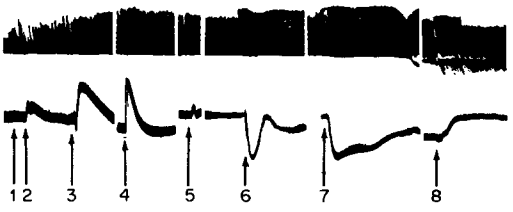

Fig. 4.—Blood pressure responses following intravenous and carotid adventitially-injected epinephrine both before and after the i. v. injection of Dibenamine in a vagotomized dog which had previously been given 50 mcg./Kg./day of reserpine for a period of ten days. Male dog, 16.3 Kg.; upper tracing: respiration; lower tracing: time in minutes. *1*, Normal tracing, B. P. 131 mm. Hg; *2*, carotids clamped, B. P. 131 to 154 mm. Hg indicating carotid reflex almost completely blocked; *3*, epinephrine, 100 γ into each carotid area, B. P. 130 to 112 to 196 mm. Hg; *4*, epinephrine, 2.0 γ/Kg., i. v., similar but steeper rise in B. P. from 110 to 205 mm. Hg; (between *4* and *5*, 25 mg./Kg. Dibenamine given i. v. over a one-hour and ten-minute period); *5*, carotids clamped $1^1/_2$ hr. post Dibenamine, B. P. 140 to 154, smaller rise than 2 but to same summit; *6*, epinephrine, 2.0 γ/Kg. i. v. as in *4*, B. P. 140 to 50 mm. Hg showing epinephrine reversal; *7*, epinephrine 100 γ in each carotid area as in *3*, B. P. 140 to 50 mm. Hg showing leakage into blood stream and reversal; *8*, vasopressin, 1.0 cc. or 2 units into each carotid area, B. P. 96 to 140 indicating leakage into blood stream with no reversal effect.

pressor action to the carotid pressoreceptors because the carotid chemoreceptors as the sole remaining compensatory nerves could neither buffer the hypotension elicited by the intravenous alseroxylon fraction nor produce hypertension after the local injection of these alkaloids. The results with reserpine in this respect are inconclusive because this alkaloid was not studied intravenously, and its relatively insoluble nature prevented a study of its local action on the carotid bifurcation.

There are three possible mechanisms by which the locally administered alseroxylon alkaloids could produce their pressor response through the carotid pressoreceptors. These are: by a local anesthetic action on the receptor nerve endings or fibers therefrom; by an adrenolytic action; or by a direct vasodilatory effect.

Using the method of Bulbring and Wajda (2) on guinea pigs, the authors investigated the possible anesthetic action of alseroxylon. However, the results were inconclusive because of an indurating effect of the acid vehicle. An anesthetic mechanism can nevertheless be ruled out because there are reports in the literature that both the alseroxylon alkaloids (3) and reserpine (4) are devoid of such an activity.

Although the alseroxylon alkaloids produced a pressor response which in some respects resembled an adrenolytic type activity, such an action can also be ruled out when the blood pressure curves following the alseroxylon fraction or epinephrine when given alone or in various sequences are carefully scrutinized. For instance, the blood pressure elevations following initial doses of alseroxylon were always of considerable magnitude while the known adrenolytic agents, Hydergine®, ergotamine, Dibenamine®, and Regitine®, (5–8) usually produce only a very slight or almost imperceptible hypertension when injected alone. Furthermore, when epinephrine was injected during the hypertension induced by a previous alseroxylon injection, either no change in the blood pressure level was observed or it slowly began to recede. In contrast, epinephrine always causes additional hypertension (a reversal of its usual action) when injected following a previous dose of an adrenolytic agent (6, 7) which itself produces a slight blood pressure elevation.

By the process of elimination, the local action of the alseroxylon alkaloids on the carotid pressoreceptors is left to a direct vasodilatory mechanism. Such a direct action on the musculature of blood vessels is not an entirely new discovery for it has frequently been postulated by other workers. However, the results of all the investigators who have studied this mode of action are still conflicting, which is most likely due to the different species of animals employed, the different Rauwolfia preparations used, and to the precise experimental techniques employed. At any rate, it is our belief that such a direct vasodilatory activity does exist, and although it is of secondary importance to the main central site of action, it is nevertheless of a great deal of importance. Thus, it has been shown that even when relatively large doses of the alseroxylon alkaloids are given intravenously or orally over a period of time, the central blocking action of these alkaloids is never quite complete. This enables the direct vasodilatory action of the alkaloids to exert a dual effect by one and the same mechanism. First, this nonneurogenic vasodilation probably contributes to the degree of Rauwolfia hypotension, the major portion of which is of the centrally-induced neurogenic variety. Secondly, the normal physiological buffering capacity of the carotid afferent nerves is potentiated by the Rauwolfia engendered muscular relaxation which occurs in the arterial walls where the receptors for these nerves are located. This physiological, as well as pharmacologically-potentiated action is of course possible only because the central pathway of this reflex arc is never completely blocked. Its importance lies in the fact that it offers an explanation as to why postural hypotension is seldom seen following Rauwolfia therapy. Accordingly, this doubly-induced buffering action limits the degree of the Rauwolfia hypotension, and in so doing it prevents any extensive venous pooling of blood. Further, when the patient assumes an erect position and the blood pressure commences to fall below the Rauwolfia-induced level, the carotid receptors further reduce their "firing off" and the blood pressure is thereby elevated or more nearly maintained at the level originally produced by the relative degree of the central sympathetic block. Thus extensive postural hypotension will not occur.

As in the preceding discussion, the results obtained in the orally treated Rauwolfia dogs again implicate the often postulated but as yet nondelimitative central nervous system as the chief locus of action for the Rauwolfia alkaloids. While the present observations yield no new clues relative to pinpointing the exact anatomical site of this central blocking action, they do definitely reaffirm that it is due to an interruption of the efferent sympathetic nervous impulses that Rauwolfia produces the major portion of its hypotensive effects. This is not only

evident from the fact that in these orally-treated animals the carotid occlusion reflex response and the usual pressor response following carotid adventitially injected alseroxylon are blocked to exactly the same degree, but is especially evident from the powerful, pressor response to carotid adventitially-injected epinephrine in these same animals.

The peculiar epinephrine pressor response was at first thought to be adrenolytic in nature. However, the pressor curve was too sharp and too transient in character for a typical adrenolytic response. Furthermore, as will be shown later, a portion of all drugs when injected into the carotid adventitia gets into the blood stream (not by accidental injection) where it may exert its typical systemic effects. With this being true also for epinephrine, then the response should have been depressor rather than pressor if the oral Rauwolfia did exert a peripheral adrenolytic action. That the epinephrine pressor response was not due to accidentally inserting the needle into or otherwise rupturing many of the minute blood vessels of the area is evident from the fact that this pressor response was never seen in any of the normal animals, but only in the orally-treated Rauwolfia dogs.

With all seemingly logical mechanisms being exhausted, it was then assumed that some of the epinephrine dosage was possibly diffusing or otherwise slowly entering the systemic circulation and thereby exerting its usual pressor action. This was eventually shown to be the case, but of even greater import is the fact that it was due only to the action of the oral Rauwolfia alkaloids that the pressor response was observed in the first place. A brief recapitulation of some of the results at this time will perhaps be of some value in clarifying the situation.

In normal animals, the injection of epinephrine into the carotid adventitia produces hypotension by constricting the arterial walls where the pressoreceptors are located. This constriction simulates hypertension in the carotid sinus; the carotid afferents increase their impulse traffic; central sympathetic substrates are inhibited; sympathetic efferent outflow is reduced; and hypotension ensues. Following similarly injected epinephrine in the oral Rauwolfia dogs, the same events transpire, but no hypotensive response is seen because of the preexisting hypotension and central sympathetic blocking action of the previously administered Rauwolfia. Instead, a potent hypertensive response is obseerved because some of the epinephrine leaks into the blood stream where it exerts its effects on the effector end organs which have become sensitized to epinephrine by virtue of a Rauwolfia-engendered, pharmacologically-induced sympathectomy. This sensitization is analogous to that seen following surgical sympathectomy. It develops over the ten-day, oral Rauwolfia feeding period and is due to the persistent reduction of sympathetic efferent impulses which in turn is due to the central sympathetic block.

That both the sensitization of the sympathetic, effector end organs as well as leakage into the blood stream are necessary for the epinephrine pressor response can be seen from the following examples: (*a*) Six hours following the intravenous administration of the alseroxylon alkaloids, the degree of hypotension and carotid reflex blocking is similar to that seen in the orally-treated dogs. However, when epinephrine is injected into the carotid area of the former animals, only a very feeble pressor or depressor response ensues. A potent depressor response is not obtained because of the preexisting hypotension and/or the central block. A potent pressor effect is also lacking because the effector end organs are not yet sensitized to the epinephrine that does reach the blood stream. (*b*) In the orally-treated dogs, carotid adventitially-injected vasopressin and ephedrine sulfate each produced a mild but persistent hypertension due to leakage into the blood stream. This pressor effect was allowed to dominate because the hypotension which these drugs always elicit in normal animals was inhibited by the Rauwolfia block on the central components of the carotid reflex arc. On the other hand, this same blocking action prevented the usual pressor response to similarly injected $NaNO_2$ so that when this drug diffused into the blood stream, only hypotension was seen.

In the last orally-treated animal, the carotid injection of epinephrine produced hypertension before Dibenamine and a mirror image hypotension after an epinephrine-reversing dose of Dibenamine. Ephedrine sulfate which cannot be reversed by Dibenamine still produced only hypertension in this animal. These results prove conclusively that Rauwolfia, unlike Dibenamine, possesses no peripheral adrenolytic action, and that portions of the dose of all drugs when injected into the adventitial layer of the carotid artery enter the blood stream to exert their usual systemic effects. If Rauwolfia possessed an adrenolytic action, the direction of the blood pressure changes following the intravenous epinephrine would have been the same both before and after Dibenamine. Likewise, if the epinephrine had not gotten into the blood stream when injected into the carotid area, the reversal would not have been observed following the Dibenamine.

The major significance of the observation that a portion of all drugs when injected or otherwise applied at the carotid area easily gain access to the blood stream is that the vasomotor changes induced in such studies must be more carefully analyzed, because the changes so induced are not brought about by a purely carotid reflex mechanism, but, instead, by the algebraic sum of the vasomotor changes mediated through the carotid reflex arc and by systemic action. Since the blood pressure changes from these two routes of administration are almost always in the opposite direction, the true potency of drugs acting locally on the carotid pressoreceptors can probably never be precisely determined. At best, only relative values may be obtained.

In conclusion, a few remarks with regard to the inactive nature of the citric acid vehicle when injected into the carotid area are in order. Previous workers have shown that a low *p*H may not affect the carotid pressoreceptors (9) as shown by action potential studies with epinephrine HCl solutions which have a *p*H of approximately 3–4 and with an HCl solution of *p*H 2.7. The inert or only feeble action of the vehicle on the carotid chemoreceptors is more difficult to explain. It is perhaps due to the fact that these receptors may already be at least partially stimulated by the respiratory depressing effects of the anesthetic and the Rauwolfia. The citric acid also may be unable to reach the chemoreceptors if these are intracellularly located which would be analogous to the permeability factors which are responsible

for the failure of certain microorganisms to take up citric acid in bacterial physiology studies on metabolism.

SUMMARY AND CONCLUSIONS

1. Results from the injection of 250 to 500 mcg. of the alseroxylon alkaloids into the adventitia of the carotid arteries at the region of the bifurcations show that these alkaloids are capable of pharmacologically potentiating the pressor buffering action of the carotid pressoreceptors against a simultaneous, centrally induced, Rauwolfia hypotension. The citric acid vehicle alone or the carotid chemoreceptors do not participate in this action.

2. This action is thought to be due to a direct vasodilatory action of the alseroxylon alkaloids upon the carotid musculature rather than to an anesthetic or adrenolytic effect. It produces vasomotor changes which are opposite in direction to similarly injected, small doses of the veratrum alkaloids. Accordingly, clinical combinations of the veratrum alkaloids and alseroxylon, while doubtless more potent hypotensively, would, by nature of the veratrum component, eliminate the potentiating, pressor, buffering action of the alseroxylon alkaloids which is important when orthostatic hypotension is considered.

3. The usual hypertension and hypotension following the respective intravenous administration of the commonly used pressor (epinephrine, vasopressin, ephedrine sulfate) and depressor agents ($NaNO_2$) are reversed to hypotension and hypertension when these drugs are injected into the adventitia of the carotid pressoreceptors of normal dogs. Further, when these same agents are injected into the carotid adventitia of oral, Rauwolfia pretreated animals, the vasomotor changes are reconverted so that the direction of the blood pressure changes are now identical to those observed following their intravenous administration. This reconverted vasomotor response is due to the blocking action of Rauwolfia on the central substrates of the carotid reflex arc together with a portion of the injected dose gaining access to the blood stream where it exerts a typical systemic effect.

4. Evidence is presented which reaffirms and extends the concept that the major hypotensive mechanism of the Rauwolfia alkaloids is through a reduction in efferent sympathetic outflow.

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Prolonged Effect of Riboflavin: Aluminum Monostearate Suspensions in Man*

By A. BRZEZINSKI, Y. M. BROMBERG, and F. G. SULMAN

The use of riboflavin suspensions with 2% aluminum monostearate for depot injection therapy has been studied in three patients. A depot of 150 mg. riboflavin takes up to 6 weeks to be absorbed, and smaller quantities take correspondingly shorter periods (100 mg., 5 weeks; 50 mg., 4 weeks). Control patients who received similar doses of riboflavin solutions showed return to normal riboflavin excretion in the urine within 3–4 days. The method of riboflavin administration in suspensions is much easier than the implantation of pellets previously described by us. The depots formed produce no local reactions and allow slow and continuous absorption of the vitamin from the site of injection.

NUTRITIONAL STUDIES in Israel have shown that riboflavin deficiency is very frequent (1) and that its incidence is much greater than that of other vitamin deficiencies (2). Clinical manifestations of this deficiency, such as glossitis, cheilitis, and corneal vascularization, are particularly common during pregnancy. Thus reduced excretion of riboflavin (averaging 95 mcg./liter of urine compared with 360 mcg./liter in normal pregnant women) was found in 21 per cent

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