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Research Articles

Pharmacological Activity of Thalicarpine

By R. A. HAHN*, J. W. NELSON, A. TYE, and J. L. BEAL

The effect of the alkaloid thalicarpine on the cardiovascular system of the anesthetized dog and on several smooth muscle preparations has been studied. In the dog, 2 mg./Kg. produced moderate pressor activity of rather long duration which was sometimes accompanied by a mild tachycardia. This effect does not appear to involve a neural pathway but may be due to a direct action either on the heart or on vascular smooth muscle. Intense, long lasting, noncholinergic hypotension was observed with doses of 10 mg./Kg. Direct depressant effects were seen on several smooth muscle preparations as well as reduction of spasmogenic effects induced by various drugs.

KUPCHAN *et al.* were the first to isolate the alkaloid thalicarpine and to describe its effect on mean arterial blood pressure of the cat (1). They reported that doses up to 5 mg./Kg. caused a transient lowering of blood pressure, while a dose of 10 mg./Kg. caused death. The hypotensive activity, in their opinion, was due to bradycardia, respiratory depression, and a weak adrenergic blocking action.

As part of a continuing study of the genus *Thalictrum* we have observed moderate pressor activity after the administration of 2 mg./Kg. of thalicarpine in the anesthetized dog. The pressor activity was sometimes accompanied by a mild tachycardia. A dose of 10 mg./Kg. was observed to produce an intense depressor response and bradycardia, with some degree of hypotension being observed for 1–3 hr. During this time of prolonged hypotension, no evidence of toxic symptoms was observed. The administration of thalicarpine to the anesthetized cat always produced a transient depressor response.

Reported here are the results of a study of the action of thalicarpine on mean arterial blood pressure of the anesthetized dog and on several smooth muscle preparations.

EXPERIMENTAL

Adult mongrel dogs of either sex were anesthetized with sodium pentobarbital (35 mg./Kg., i.p.). After surgical anesthesia was achieved the trachea was cannulated and bilateral cervical vagotomy performed. The right carotid artery was cannulated and blood pressure recorded *via* a mercury manometer on a kymograph. The right femoral vein was then cannulated with a 3-in. length of polyethylene tubing for the injection of drug solutions. When infusions were administered the left femoral vein was cannulated and the infusion given by means of a Harvard infusion pump. Heart rate was recorded by means of a Sanborn Twin Viso-

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recorder. In those dogs which were pretreated with reserpine (1 mg./Kg., i.p., 24 hr. prior to experimentation) the dose of sodium pentobarbital was reduced to 20 mg./Kg. (2).

Adult cats of either sex were anesthetized with sodium pentobarbital and prepared in a similar manner.

Helically cut 3-cm. strips of descending thoracic rabbit aorta were prepared according to the method of Furchgott (3). The tissue was bathed with Krebs bicarbonate solution and gassed with a mixture of 95% oxygen and 5% carbon dioxide in a 10-ml. tissue bath at a constant temperature of 37°. Movements were recorded isotonically on a slow moving kymograph by means of a frontal lever, with a magnification of 1:10 and a tension of 2 Gm. After mounting of the tissue, 1.5 hr. were allowed for equilibration before the addition of drugs. Two-centimeter lengths of adult rabbit ileum and uteri of adult rats were set up in a similar apparatus. These tissues were bathed with Tyrode's solution and 0.5 hr. was allowed for equilibration before the addition of drugs. A resting tension of 2 Gm. was used with the rat uterus, while light tension was used in the case of rabbit ileum.

Solutions of thalicarpine were prepared in physiological saline with the aid of dilute hydrochloric acid, those of dibenamine in polyethylene glycol, while all other drug solutions were prepared in physiological saline. Injection volumes administered to *in vivo* preparations never exceeded 1 ml. and were followed by a 1-ml. flush of saline, while drug solutions added to *in vitro* preparations never exceeded 0.2 ml. All drug solutions were prepared fresh on the day of the experiment. All doses refer to mg. or mcg. of the parent compound. Thalicarpine[1] was isolated and purified according to the method of Tomimatsu *et al.* (4).

Each observation represents a minimum of 3 experiments.

The following drugs were used: thalicarpine, dibenamine, reserpine phosphate, norepinephrine bitartrate, epinephrine bitartrate, dichloroisoproterenol hydrochloride, isoproterenol hydrochloride, dimethylphenylpiperazinium iodide, atropine sulfate, histamine phosphate, tripelennamine hydrochloride, methacholine chloride, and vasopressin.

RESULTS

Figure 1 shows the effect of administration of 2 mg./Kg. of thalicarpine to the anesthetized, vagotomized dog. A pressor response with gradual onset occurred with a peak mean value of +29.2 ± 2.0 mm. of Hg (mean ± standard error) with some degree of hypertension lasting for 10–35 min. Occasionally the pressor response was accompanied by a mild tachycardia. Some animals responded to this dose of thalicarpine in a biphasic manner; a slight, short-lived fall in blood pressure followed by the sustained pressor response. Further administration of this dose of thalicarpine was ineffective in producing a pressor response, and only transient depressor activity was seen. Doses of thalicarpine less than 2 mg./Kg. did not produce any demonstrable effect on arterial blood pressure.

[1] Its identity as thalicarpine was established by infrared comparison and mixed melting point determination with an authentic sample kindly supplied by Dr. S. M. Kupchan.

Upon the injection of 10 mg./Kg. of thalicarpine to the anesthetized vagotomized dog, a prompt intense depressor response occurred (Fig. 2). The maximal depressor response had a mean value of −112.5 ± 8.5 mm. of Hg and some degree of hypotension was observed for 1–3 hr. Bradycardia (30–40 beats/min.) accompanied this depressor activity. Despite this precipitous fall in blood pressure, no evidence of overt toxic effects was observed, the EKG and respiratory patterns being the same as observed before the injection. Doses of thalicarpine between 5 and 10 mg./Kg. produced increasing depressor activity.

Doses of thalicarpine up to 5 mg./Kg., when given to the anesthetized cat, produced only transient depressor activity. Pressor activity was never seen upon either the initial or subsequent injections.

In an attempt to determine the mechanism by which thalicarpine brought about the initial pressor response in the dog at the 2 mg./Kg. dose level, several experiments were conducted.

To determine if the release of endogenous catecholamines was necessary for the response, dogs were pretreated with reserpine (1 mg./Kg., i.p., 24 hr. prior to experimentation). A typical response of the reserpinized dog to 2 mg./Kg. of thalicarpine is shown in Fig. 3. Reserpine pretreatment did not prevent the initial pressor activity. Furthermore, when the initial pressor activity of thalicarpine had been demonstrated and further injections produced only a fall in blood pressure, the infusion of norepinephrine (1 mg. in 30 min.) did not return the ability of thalicarpine to increase arterial blood pressure. Dibenamine pretreatment (10 mg./Kg.), which abolished the pressor activity of injected epinephrine, had no effect on the pressor activity of thalicarpine.

To ascertain if augmentation of cardiac activity played a part in the pressor effect of thalicarpine, dogs were pretreated with dichloroisoproterenol (DCI). Although this pretreatment effectively abolished control responses to isoproterenol, thalicarpine still produced a pressor response (Fig. 4). DCI was also ineffective in preventing the depressor response to subsequent injections of thalicarpine.

A ganglionic stimulant action on the part of thalicarpine would not appear to account for the pressor action in view of the results obtained with dibenamine or with DCI. These drugs are known to be α- and β-adrenergic receptor blocking agents, respectively, and since neither agent blocked the response, a neural pathway does not seem to be

Fig. 1.—The response of the anesthetized, vagotomized dog to 2 mg./Kg. of thalicarpine. The initial injection produced a pressor response, while a subsequent injection caused only a fall in blood pressure. Key: blood pressure in mm. of Hg; heart rate indicated by numbers above blood pressure tracing (beats/min.); T, thalicarpine.

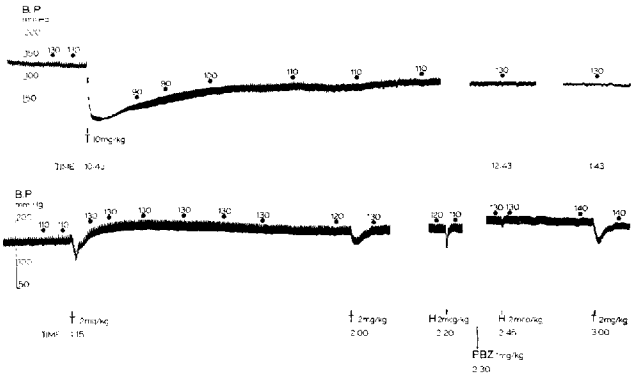

Fig. 2.—The response of the anesthetized, vagotomized dog to 10 mg./Kg. of thalicarpine. Key: blood pressure in mm. of Hg; heart rate indicated by numbers above blood pressure tracing (beats/min.); T, thalicarpine.

Fig. 3.—The response of the anesthetized, reserpinized dog to 2 mg./Kg. thalicarpine. Key: blood pressure in mm. of Hg; heart rate indicated by numbers above blood pressure tracing (beats/min.); T, thalicarpine; H, histamine; PBZ, tripelennamine.

involved. In addition, no augmentation of dimethylphenylpiperazinium (DMPP)-induced pressor activity was observed when DMPP (25 mcg./Kg.) was injected during a typical pressor response to thalicarpine.

The depressor action of repeated injections of thalicarpine at the 2 mg./Kg. dose was not blocked by atropine pretreatment, although the depressor effect of injected acetylcholine was completely abolished (Fig. 4). Vagotomy also did not alter the response. The possibility of histamine release was ruled out when pretreatment with tripelennamine did not block the thalicarpine effect, although injected histamine was prevented from causing its usual depressor response in the dog (Fig. 3).

The precipitous and long lasting hypotensive response to 10 mg./Kg. of thalicarpine was not altered by cervical vagotomy or atropine pretreatment. DMPP-induced pressor responses, known to be mediated through ganglionic stimulation, were the same during the prolonged hypotension produced by thalicarpine as they were at preinjection times. Similarly injected epinephrine (2–4 mcg./Kg.) produced the same rise in blood pressure during the intense thalicarpine hypotension as it did during preinjection times.

When thalicarpine was added to a bath with isolated rabbit ileum a prompt relaxation occurred. Upon washing, this effect was abolished and normal activity resumed. When methacholine chloride was introduced the characteristic spasm was seen; however, pretreatment with thalicarpine greatly reduced the methacholine-induced spasm of the ileum. This effect became less with time (Fig. 5). Normal contractions of the rat uterus were inhibited both in frequency and amplitude upon the addition of thalicarpine into the bath. Vasopressin-induced spasms of the uterus were completely blocked after thalicarpine. With repeated washing of the tissue the vasopressin effect returned (Fig. 6). Similar results were obtained on epinephrine-induced contractions of the rabbit aortic strip. After pretreatment with thalicarpine, in concentrations having no effect of their own (10–40 mcg./ml.), contractions

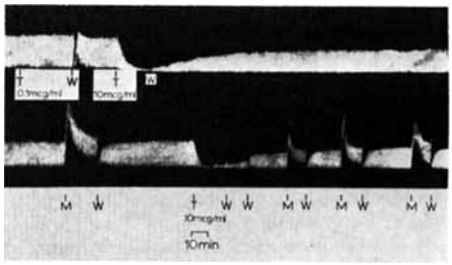

Fig. 5.—Continuous kymograph recording of the isolated rabbit ileum. The administration of thalicarpine caused a prompt relaxation of the ileum. Pretreatment with thalicarpine greatly reduced the methacholine-induced spasm of the ileum. Key: T, thalicarpine; M, methacholine 0.05 mcg./ml.; W, wash.

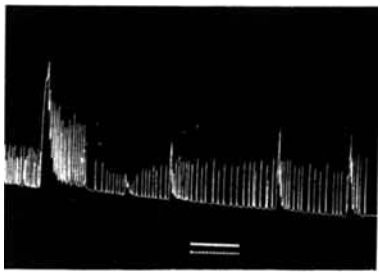

Fig. 6.—Kymograph recording of the isolated rat uterus. The administration of thalicarpine inhibited normal contractions of the uterus and greatly reduced the vasopressin-induced spasm of the uterus. Key: P, vasopressin 0.1 unit/ml.; T, thalicarpine 40 mcg./ml.; W, wash.

Fig. 4.—The response of the anesthetized, vagotomized dog to 2 mg./Kg. thalicarpine. Key: blood pressure in mm. of Hg; heart rate indicated by numbers above blood pressure tracing (beats/min.); Is, isoproterenol; DCI, dichloroisoproterenol; T, thalicarpine; Ach, acetylcholine; A, atropine.

due to epinephrine were greatly reduced, the effect being reversed with time and repeated washing of the tissue.

DISCUSSION

The administration of 2 mg./Kg. of thalicarpine to the anesthetized vagotomized dog produced a fairly long lasting pressor response with a gradual onset. If liberation of endogenous catecholamines was necessary for the response, then pretreatment with reserpine should block this action, since reserpine is known to deplete sympathetically innervated tissue of catecholamine stores and, thus, reduce the response of various organs to sympathetic nerve stimulation and to indirectly acting sympathomimetic amines (5–7). Thalicarpine-induced pressor activity was observed in the reserpinized dog, and thus, liberation of catecholamines does not appear necessary for the response. When the initial pressor activity to thalicarpine had been demonstrated and further administration of thalicarpine produced only depressor activity, norepinephrine infusion could not restore the pressor action. When the α-adrenergic receptor blocking agent, dibenamine, was given in a dose sufficient to block the pressor effect of injected epinephrine, the pressor activity of thalicarpine was not altered. Therefore, the increase in arterial blood pressure due to thalicarpine is not due to release of catecholamines or a direct α-receptor stimulant effect.

In dogs pretreated with DCI, thalicarpine pressor activity was observed, although positive chronotropic activity of control injections of isoproterenol was blocked (Fig. 4). DCI has been demonstrated to block the positive chronotropic and inotropic effects of adrenergic stimuli in dogs, while it is ineffective against similar responses produced by digoxin or theophylline (8).

Since the pressor activity of thalicarpine does not appear to be due to an epinephrine-like effect, the possibility exists that the effect may be similar to the cardiac action of digoxin which is not altered by DCI. Papaverine also has direct positive inotropic effects (9), and if one views the structure of thalicarpine as reported by Kupchan and Yokoyama (10), it is apparent that thalicarpine contains the basic moiety of papaverine. The possibility exists, therefore, that the pressor activity of thalicarpine may be due to a direct positive inotropic effect to increase cardiac output, especially since a positive chronotropic action was not always seen with thalicarpine.

Some transient direct stimulant effect on vascular smooth muscle might also be a possibility as the cause of the pressor action, although bath concentrations of thalicarpine up to 40 mcg./ml. produced no contraction of the isolated rabbit aortic strip. Vasopressin has been shown to be a potent vasoconstrictor in the intact animal but without effect on rabbit aortic strips (11); a similar situation may exist in the case of thalicarpine.

The rise in blood pressure produced by injections of DMPP during typical thalicarpine hypertension was of the same magnitude as that produced by control injections. DMPP has been shown to be a ganglionic stimulant (12), and if thalicarpine also possessed this activity, then an additive pressor action might be expected. Such was not the case and, therefore, ganglionic stimulation as a basis for the pressor effect of thalicarpine does not appear likely. Furthermore, if ganglionic stimulation is a factor a neural pathway would be involved and dibenamine, DCI, or reserpine pretreatment should have blocked the response.

The hypotension induced by repeated injections of 2 mg./Kg., or by a single injection of 10 mg./Kg. of thalicarpine, does not appear to be cholinergic in nature for atropine did not alter this effect, although it effectively blocked injected acetylcholine (Fig. 4). Cervical vagotomy also had no effect on this response to thalicarpine.

It has been reported that 10 mg./Kg. of thalicarpine caused death in the anesthetized cat (1). In our experiments the administration of this dose of thalicarpine to the dog, and doses of 5 mg./Kg. in the cat, never produced overt signs of toxicity, or death. Animals receiving this dose of thalicarpine appeared normal with respect to EKG and respiratory patterns, and in general appearance. Although the depressor response was intense and long lasting, the blood pressure did return to approximately preinjection levels (Fig. 2). We do not exclude the possibility, however, that this depressor effect may represent thalicarpine toxicity.

The observations that epinephrine and DMPP produced similar pressor effects during thalicarpine hypotension to those at preinjection times indicate that adrenergic blockade or ganglionic blockade are not prime causes of the potent hypotension.

Thalicarpine, in bath concentrations of 10–40 mcg./ml., produced a prompt relaxation of the isolated ileum and uterus. Spasms of the ileum produced by methacholine and those of the uterus produced by vasopressin, were greatly reduced by thalicarpine pretreatment (Figs. 5 and 6). Thalicarpine pretreatment of the isolated rabbit aortic strip also greatly inhibited epinephrine-induced contractions. This effect was reversed on all 3 preparations with time and repeated washings of the tissues. Thus our results indicate a nonspecific inhibition of smooth muscle activity and an ability to antagonize spasmogenic effects of drugs of diverse nature on various smooth muscle as an important pharmacological effect of thalicarpine. This peripheral inhibitory effect of smooth muscle plus bradycardia may well be the cause of the potent hypotension observed with higher doses of thalicarpine.

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