Release of Catecholamines by Vasopressin into the Cavernous Sinus Blood of the Dog. (31603)

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In most instances, the hemodynamic response to pressor drugs is a rather predictable phenomenon. One perplexing exception, however, is the blood pressure response to exogenous vasopressin. It has been noted that doses of vasopressin which, in some dogs, cause considerable increase in systemic blood pressure, may in others be totally ineffective. This great variability of the pressor effect of vasopressin between dogs may be due to the fact that it is not mediated by vasopressin alone. This report deals with that problem and presents evidence that exogenous vasopressin causes acute liberation of catecholamines from the brain of the anesthetized dog in sufficient amounts to influence the cardiovascular system(1).

Materials and methods. Mongrel dogs of either sex, between 8 and 25 kg, were anesthetized with 35 mg/kg sodium pentobarbital. All injections were made through an exposed, cannulated femoral vein and the mean blood pressure was recorded with a Statham transducer, P23AA, on a Beckman Type RB Dynograph® recorder. A cannula for the collection of cavernous sinus blood was placed by excising the soft palate with a cautery. After removal of the mucous membrane which covers the palatine bone structure, a dental drill, fitted with a No. 8 burr in a contra-angle head was used to make an opening through the palatine bone structure, in close proximity to the sella turcica, down to the venous tissue surrounding the cavernous sinus. This opening was made in such a way as to allow a snug fit of PE 260 Intramedic® polyethylene tubing in the outer, more dense, osseous layer. Beneath the dense osseous layer, an undercut was formed by the removal of about a third of the cancellous layer. This allowed for the mushrooming of the soft wax used to secure the cannula in place. When the burr-hole was completed and the animal had been injected with 10,000 units of heparin intravenously, a portion of the venous tissue surrounding the cavernous sinus was carefully torn with a burr. Care was taken to insure that no arteries were broken during this procedure. When proper venous blood flow was achieved, the cannula was positioned, and the soft wax was forced into the burr-hole and around the cannula with a ring-end flat soldering lug previously slipped around the cannula. After placement of the cannula, 4 control samples of cavernous sinus blood were drawn at approximately one-minute intervals. A single 300 milliunits per kilogram (mU/kg) dose of vasopressin (Parke-Davis Pitressin®) was then injected through the exposed femoral vein and 5 ml samples were collected in polypropylene test tubes from the cavernous sinus at intervals throughout the entire pressor response to vasopressin. The collection of samples was governed by the pressor response itself rather than precise time intervals because of the great variability between dogs in the response to intravenously injected vasopressin. After collection, the samples were centrifuged at 12,000 $\times$ *g* for 20 minutes at 4°C to insure complete removal of all particles and platelets from the plasma. Two milliliters of whole plasma were removed and analyzed for total catecholamine content.

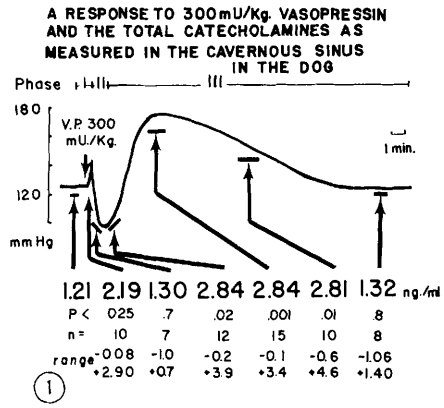
A second group of 15 mongrel dogs was anesthetized and arranged to record arterial pressure. Three control responses to 300 mU/kg of vasopressin were recorded in the manner described previously. However, after the control responses to 300 mU/kg of vasopressin had been recorded, the circulation to the central nervous system was occluded and 3 subsequent responses to vasopressin were recorded. The area of the response to vasopressin was then measured in arbitrary units with a planimeter.

The analysis used for determination of total catecholamine was a combination of the alumina absorption technique of Anton and Sayer as applied to a column and the oxidation of the catecholamines to their

fluorescent, lutin form by Häggendal(5,6). The necessity of using small volumes of blood to allow repeated sampling required minimum fluorescent background. This was largely achieved by reduction of the background from the aluminum oxide. The aluminum oxide used in the procedure was prepared by heating the aluminum oxide in a Vycor® column to 1000°C while fluidizing the bed with a stream of argon. After reaching the desired temperature, it was then exposed to a flow of elemental chlorine for 4 hours after which the heating and chlorine were discontinued. After cooling the aluminum oxide in the argon stream, it is free of fluorescent materials or contaminants which act as quenching agents during the oxidation of the catecholamine for the fluorescent analysis. All fluorometric readings were made on a Turner Model III Fluorometer® fitted with a #110-865 high sensitivity conversion kit in 12 × 75 mm Pyrex® test tubes, matched for fluorescent background.

Results. The average phase I response in 15 dogs shown in Fig. 1 was an increase from 127 mm Hg in the control period to an average of 145 mm Hg and was initiated at an average of 5 seconds after intravenous injection of vasopressin 300 mU/kg in the femoral vein. The average depressor effect in phase II was to 95 mm Hg at the end of the first minute. The more prolonged pressor effect of phase III peaked between 4.5 and 5.5 minutes at an average of 172 mm Hg, with the average duration of the responses at 18 minutes. The average catecholamine control level for the 15 dogs as measured in cavernous sinus blood was 1.21 ng/ml of whole plasma. During the phase I pressor response to vasopressin the catecholamine level rose to 2.19 ng/ml. During phase II the catecholamine level was 1.30 ng/ml and at the beginning of phase III immediately after phase II the catecholamine level rose to 2.84 ng/ml ($p < 0.02$). At the peak of phase III the catecholamine level was 2.84 ng/ml ($p < 0.001$) and at the response half-life, the catecholamine level was 2.81 ng/ml ($p < 0.01$). At the termination of phase III the catecholamine level was 1.32 ng/ml.

Continuous catecholamine release into the cavernous sinus blood was not, or could not be measured, thus, total amounts of catecholamines from the brain could not be determined. If, however, spot samples which contained the highest concentration of catecholamines are compared to the maximum blood pressure during the phase III response, a trend is demonstrated toward a correlation between the extent of the pressor response and the catecholamines secreted from the brain (Fig. 2).



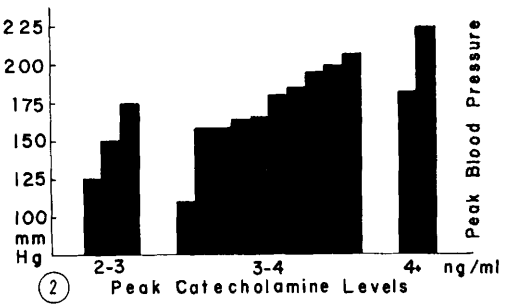


FIG. 1. The method of statistical analysis used was a paired comparison on the same individual between pretreatment and post-treatment levels. The T test supports the conclusion of significant differences. The data on range of values show that some dogs developed very marked increments of catecholamine levels.

FIG. 2. A comparison of peak pressor responses and peak catecholamine concentrations in individual dogs.

Ganglionic blockade induced with hexamethonium did not prevent the usual release of catecholamines, although mean blood pressure was reduced and no appreciable further fall was caused by vasopressin.

Four dogs received 50 mU/kg vasopressin. Two showed slight pressor responses and

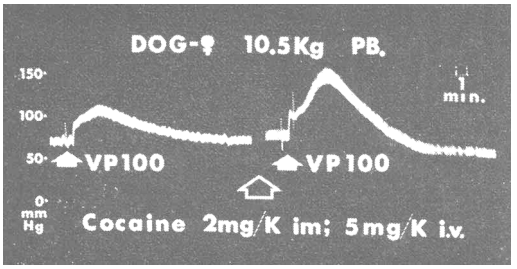


FIG. 3. 100 mU/kg vasopressin was injected into the pentobarbital anesthetized dog after which cocaine 2 mg/kg intramuscularly and 5 mg/kg intravenously was injected. The potentiation by cocaine is demonstrated in the subsequent response to 100 mU/kg vasopressin.

slight output of catecholamines. Two showed neither, so that this is the approximate minimally effective dose under these conditions.

Marked potentiation of vasopressin 100 mU/kg by cocaine is shown for the dog in Fig. 3. In those experiments where the circulation to the central nervous system was occluded it was demonstrated that there is a sharp decrease in the pressor effect of 300 mU/kg of vasopressin intravenously (Table I).

Discussion. By using the heat-chlorine treated Al_2O_3 in the catecholamine analysis, the acute release of central nervous system catecholamines was observed. The actual amount of catecholamines was widely variable as can be seen in Fig. 1. However, by removing the source of catecholamines by occlusion of the circulation to the central nervous system the pressor response was diminished and in some cases, eliminated. The potentiation of the pressor response to vasopressin by cocaine can best be attributed to the classical cocaine potentiation of catecholamines. The variability, therefore, of the pressor response to exogenous vasopressin appears to be largely associated with the amount of catecholamines released from available stores in the central nervous system. The release of catecholamines in phase III was not associated with fall of systemic pressure in phase II and was not affected by ganglionic blockade and may, therefore, be directly mediated. The synergism between vasopressin and catecholamines(2,3) and the interaction of vasopressin with adrenergic drugs and systems(4) takes on a new aspect in view of the fact that catecholamines are released by vasopressin itself; nevertheless, the potentiation of vasopressin by alpha adrenergic blocking agents remains unexplained(3,7,8).

Summary. The highly variable pressor response to exogenous vasopressin was studied in pentobarbital anesthetized dogs. It was demonstrated that exogenous vasopressin causes a release of catecholamines from stores in the central nervous system as measured in the cavernous sinus blood. When the circulation to the central nervous system is occluded the pressor response to exogenous vasopressin is greatly reduced.

TABLE I. Vasopressin Responses in Dogs After Occlusion of the Circulation to the Central Nervous System.

Control responses* V.P., 300 mU/kg I.V.				Responses after occlusion* V.P., 300 mU/kg I.V.		
1	2	3	Method of occlusion	1	2	3
3.0	2.5	2.3	Pressure[1]	0.7	0.4	0.3
3.08	2.37	2.02	Pressure[1,4]	1.21	1.08	0.60
2.4	2.1	1.9	Pressure[1,4]	0.45	—	—
2.2	1.4	1.7	Pressure[2,4]	<0.1	<0.1	<0.1
2.8	1.9	1.45	Pressure[2]	0.15	<0.1	0.15
1.1	1.0	1.2	Decapitation[4]	0.5	0.5	0.5
3.91	3.93	2.83	Cavernous sinus occlusion[3]	1.8	0.61	0.35
4.38	4.29	4.13	Cavernous sinus occlusion[3]	<0.1	<0.1	<0.1
3.48	2.25	1.41	Cavernous sinus occlusion[3]	0.53	0.82	0.61

* Area of pressor response measured in arbitrary units with a planimeter.

[1] Pressure created with modified vise.

[2] Pressure created with screw-type tubing clamps.

[3] Block created by plugging cavernous sinus with soft wax after surgical exposure of the area.

[4] Artificial respiration.

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Estrogen Antagonisms: Relationship Between Estrogen Antagonistic And Progestational Potencies of Δ^4-3-Oxosteroids. (31604)

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The relationship between estrogen antagonistic and progestational effects of various Δ^4-3-oxosteroids has been the subject of speculation for some time(1,2,3). Discussing various 17-substituted relatives of 19-nortestosterone, Edgren *et al*(1) stated: " . . . the absence of a direct glandular effect of 19-nortestosterone, an antagonist, and conversely, the absence of estrogen antagonistic action of the butyl, which is still an active progestin, suggest that the activities are separated." Some lack of correspondence beween progestational and anti-estrogenic effects was also observed among the derivatives of 17α-acetoxyprogesterone(2). Since these observations were at best suggestive, it seems germane at this point to examine in detail the relationships of these activities in a number of newer materials. Such considerations are crucial at this time because of the recent clinical interest in estrogen antagonists as contraceptives, acting at the level of cervical mucus(4).

Materials and methods. Estrogen antagonistic potencies were determined in a mouse vaginal smear test following the protocol of Edgren(5). Spayed mice received estrone at a standard dose of 0.5 μg per day for 4 days. Vaginal smears were examined on the afternoon of day 5; those showing an estrogenic vaginal response (absence of leukocytes) were returned to the colony and the remaining mice were reexamined on the morning of day 6. Progesterone was employed as a standard, and compounds were compared to progesterone at doses estimated to reduce by 50% the expected response to the estrogen. Normally, in this test, the standard dose of estrone produced 80-100% positive responses in treated mice, and the ED_{50} for progesterone was approximately 400 μg.

Progestational potencies were estimated from a Clauberg test(6). Intact female rabbits, weighing about 1 kg, were primed daily for 6 days with 5 μg of estradiol-17β. Test compound injections were initiated on the day following the final priming injection and continued for 5 days. On the day after the final injection the rabbits were sacrificed and uterine segments were removed from each for histological examination. The uteri were scored according to the McPhail index(7). Progesterone, again the standard, normally produced an average McPhail index of +2 at a daily dose of about 100 μg. Test compounds were compared at the doses estimated to produce a +2 McPhail index.

The present communication will examine simultaneously the progestational and anti-estrogenic potencies of several series of closely related delta-4-3-oxosteroids that have both types of activity and that are potent, or are related to compounds that are potent, in one or the other test.

Results and discussion. Included in the group of compounds chosen for comparison are some of the most potent progestins and estrogen antagonists that have been studied in this laboratory (Table I). 19-Norproges-