Mechanism by Which Serotonin, Norepinephrine and Reserpine Cause Central Vasomotor Inhibition

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In previous experiments, we found that serotonin, norepinephrine and their respective precursors, 5-hydroxytryptophan (5HTP) and 3,4-dihydroxyphenylalanine (DOPA), had qualitatively the same cardiovascular effects as reserpine when they were injected into a cerebral lateral ventricle of anesthetized and unanesthetized dogs. The results were all consistent with the premise that the acute cardiovascular effects of reserpine are mediated centrally by serotonin and/or norepinephrine, either released from a bound and inactive to a free and active form or formed from their respective amino acid precursors.

Marrazzi and Hart and Slocombe, Hoagland and Tozian demonstrated that both agents transiently block cerebral synaptic transmission when injected into a carotid artery. The mechanism is not known with certainty. Among several possibilities are a direct effect on neurohumoral transmitter substances, a direct effect on synaptic receptors, interference with specific enzyme systems and an indirect effect resulting from change in blood flow. Since serotonin and norepinephrine are both strong vasoconstrictor agents, the present experiments were designed to investigate whether vasoconstriction might contribute in some manner to their ability to cause central vasomotor inhibition.

Methods

Adult mongrel dogs, weighing from 9 to 19 Kg., were anesthetized with morphine sulfate (2 mg./Kg. i.m.) followed in 20 minutes by sodium pentobarbital (15 mg./Kg. i.v.). Both vagus-sympathetic-depressor trunks were cut and intermittent positive pressure respiration employed throughout all experiments. Mean arterial pressure was recorded on a smoked drum by a mercury manometer connected to a cannulated femoral artery. Heart rate was recorded simultaneously on the same smoked drum by a method described previously.

A cannula was implanted in a cerebral lateral ventricle of 27 dogs according to the technic described by Feldberg and Sherwood in order to facilitate central injection of drugs dissolved in from 0.2 to 0.5 ml. of physiologic saline. Injection of these volumes of saline alone did not affect arterial pressure or cardiovascular reflexes.

In order to change the temperature of the cerebrospinal fluid in 25 dogs, a needle or soft polyethylene cannula was passed into the cisterna magna and connected by means of polyethylene tubing containing physiologic saline (volume approximately 2 ml.) to a cannula placed in a lateral ventricle. Cerebrospinal fluid was circulated from the cisterna magna to the lateral ventricle with the aid of a finger-type pump at a rate of from 1.2 to 2.0 ml./min. Warming was accomplished by placing the polyethylene tubing containing the circulating cerebrospinal fluid in a water-bath heated to 42 to 48 C. and cooling by lowering the temperature of the water-bath from 10 to 18 C.

Experiments in which active bleeding into the cerebrospinal fluid occurred were discontinued and are not included in this report.

In experiments on unanesthetized dogs a cannula was placed in a lateral ventricle several days prior to the experiment; several weeks beforehand a common carotid artery was explanted into a tube of skin, the contralateral carotid sinus denervated and both aortic depressor nerves cut by employing the expedient of identifying the vagus just below the level of the carotid sinus and then cutting the remainder of the vagus-sympathetic-depressor trunk. These procedures permitted painless measurement of the carotid occlusion response by compression of the explanted tube of skin. Local anesthesia was used in cannulation of a femoral artery.

Results

As in previous experiments, serotonin, norepinephrine and their respective precursors injected into a lateral ventricle or the cisterna magna reproduced the effects of reserpine given in the same manner or intravenously.
The effects consisted of hypotension, bradycardia and marked inhibition of the pressor response to occlusion of the common carotid arteries (as illustrated in figs. 3 and 4, referred to below). Our initial experiments were directed toward determining whether other vasoconstrictor agents might produce the same effects.

Central Effects of Angiotensin and Vasopressin

Angiotensin (5 to 100 units, 1 unit having approximately three-fourths the pressor activity of 1 μg. of norepinephrine in dogs anesthetized with sodium pentobarbital) was injected into a lateral ventricle of each of 5 anesthetized dogs. Heart rate was little affected, and there was usually no decrease of arterial pressure. It caused 14 to 55 per cent decrease of the carotid occlusion response (average 26 per cent). The inhibitory effect appeared within 20 minutes after injection (fig. 1).

Vasopressin (1 to 8 units) was injected into a lateral ventricle of each of 4 anesthetized dogs. One unit was without effect; 2 or more units caused slight to moderate reduction of the carotid occlusion response, the average reduction being 32 per cent. The inhibitory effect appeared after a latent period of approximately 10 minutes and was maximum after from 20 to 30 minutes. Heart rate was unchanged or slightly slowed and, as after injection of angiotensin, arterial pressure was unchanged or slightly elevated.

Central Effects of Vasodilator Drugs

Since vasoconstrictor drugs of widely differing structure, such as norepinephrine, serotonin, angiotensin and vasopressin, all inhibit the reflex response to carotid occlusion when they are given centrally, the implication is that vasoconstriction per se is accountable. If central inhibition does depend on local vasoconstriction, vasodilator drugs should have an opposing action. Accordingly, sodium nitroprusside (100 to 500 μg.), a powerful vasodilator agent, was injected into a lateral ventricle or the cisterna magna of each of 9 anesthetized and 2 unanesthetized dogs in which arterial pressure and the carotid occlusion response had been depressed by prior injection of 5HTP, DOPA, norepinephrine or reserpine. There was temporary recovery of response to carotid occlusion in all experiments (fig. 2). Restoration of arterial pressure and heart rate was less pronounced than that of the carotid occlusion response. The effect appeared within 10 minutes, reached a maximum between 15 to 30 minutes, and then decreased. With large dosage some effect continued for more than one hour.

Although temporary, the restoration of the carotid occlusion response was nearly complete in some experiments. In others, when existing central inhibition was not marked, recovery was complete and permanent. In control experiments of the same duration and employing the same dosages of central inhibitory drugs, spontaneous recovery was not observed. When the course of the experiment was reversed, with injection of nitroprusside preceding injection of a vasoconstrictor agent, central inhibition was less pronounced.

When central inhibition due to norepinephrine or 5HTP was intensified by prior central injection of an amine oxidase inhibitor, JB 516 (β-phenylisopropylhydrazine), injection of nitroprusside, as in previous experiments, had a slight or no modifying effect.

Histamine (100 to 500 μg., measured as
phosphate), injected into a lateral ventricle of 17 anesthetized dogs and 1 unanesthetized dog, in which the carotid occlusion response had been inhibited by prior injection of 5HTP, DOPA, angiotensin, vasopressin or reserpine, had essentially the same restorative effects as did nitroprusside (figs. 1 and 3), but its action was less powerful and of shorter duration. Effects were maximum within from 5 to 15 minutes and usually disappeared within 30 minutes.

Central Effects of Warming and Cooling Cerebrospinal Fluid

Since all of the vasoconstrictor drugs tested had a central inhibitory action that was opposed by either of the vasodilator drugs employed, and since cooling of blood or body tissues usually produces vasoconstriction and warming produces vasodilatation, the effect on central vasomotor activity of cooling and warming the cerebrospinal fluid was measured.

Continuous withdrawal of spinal fluid from the cisterna magna into plastic tubing, and circulation of it back into a lateral ventricle at a rate of 2 ml./min. with the aid of a finger-type pump, did not produce measurable change in arterial pressure, heart rate or the carotid occlusion response when the ambient room temperature was approximately 25 C. When the plastic tubing containing the circulating cerebrospinal fluid was immersed in a bath cooled to 10 to 18 C, for from 10 to 30 minutes, arterial pressure declined on an average of 17 mm. Hg, and the carotid occlusion response was reduced on an average of 35 per cent in 5 dogs. There was also a slight decrease of heart rate. When cooling was discontinued, arterial pressure and heart rate and occlusion response usually returned to control values within 20 to 30 minutes.

Passage of the circulated cerebrospinal fluid through a bath warmed to 42 to 48 C. caused no definite change in arterial pressure, heart rate or the occlusion response. When cerebrospinal fluid was warmed after central inhibition had been produced by injection of 5HTP, DOPA, norepinephrine or reserpine, the procedure had the same effect as administration of a vasodilator drug (fig. 3). Restoration of arterial pressure, heart rate and the occlusion response appeared within 5 minutes and was maximum after 10 to 20 minutes of warming. Recovery of the occlusion response, while prominent, was usually incomplete and less than that following injection of a vasodilator drug. The effect of warming started to disappear promptly after removal of the tubing from the bath and disappeared completely within from 10 to 20 minutes, central inhibition then being as marked as before warming.

When arterial pressure and the occlusion response had been reduced by cooling the cerebrospinal fluid, injection of histamine into the circulating fluid markedly opposed the inhibitory effect (fig. 3). In dogs in which partial central inhibition had been produced by central injection of norepinephrine or 5HTP, cooling of cerebrospinal fluid caused further inhibition.

Central Effects of Adrenergic and Serotonergic Blocking Agents

In 5 anesthetized dogs, phentolamine, lysergic acid diethylamide (LSD) or its brom derivative (BOL) was administered into a lateral ventricle after central inhibition had been produced by norepinephrine or 5HTP. Phentolamine (5 mg.) caused temporary res-
Figure 3

Effect of injection of 5HTP into a lateral ventricle of anesthetized dog and opposing effect of warming of cerebrospinal fluid (CSF), followed by opposing action of histamine on inhibitory effect of cooling of CSF. N, norepinephrine 5 µg. i.v.; S, serotonin 60 µg. base i.v. Time marks: 1 minute.

Discussion

In previous experiments, as here, it was found that serotonin, norepinephrine and their respective amino acid precursors, like reserpine, produce hypotension, bradycardia and inhibition of the pressor response to occlusion of the common carotid arteries when they are injected into a lateral ventricle. These observations suggest the possibility that the centrally mediated cardiovascular effects of reserpine may depend on release of serotonin and/or norepinephrine from a bound form within the brain to a free and active one. The present experiments demonstrate additionally that these inhibitory effects can be opposed or abolished by injecting a vasodilator drug into a lateral ventricle or by warming the cerebrospinal fluid.

Other vasoconstrictor agents (angiotensin and vasopressin), given centrally, also inhibited the carotid occlusion response, but their activity was less than that of serotonin and norepinephrine, and they failed to lower arterial pressure despite inhibition of the carotid occlusion response. It was assumed that their lesser activity was due to slower diffusion and penetration into brain tissues because of their larger molecular size.

The opposing actions of vasoconstrictor and vasodilator drugs and of warming and cooling the cerebrospinal fluid suggest that these actions may be mediated through change in blood supply to areas of the brain concerned with vasomotor activity. This hypothesis was not susceptible to proof by the technic employed, but all of the experimental results are in accord with it. It has been demonstrated experimentally many times that vasomotor centers are extremely sensitive to ischemia, and clinically, insufficiency of cerebral blood supply accompanying thrombosis of an internal carotid artery is associated with
Effect of injection of norepinephrine into a lateral ventricle of anesthetized dog and opposing action of Regitine (phentolamine). N, norepinephrine 5 μg i.v.; S, serotonin 60 μg in i.v. Time marks: 1 minute.

decline of systemic arterial pressure. Aviado et al. demonstrated the existence of both inhibitory and excitatory intracranial receptors which are activated by potassium and veratridine and which affect arterial pressure, heart rate and respiration. Intracisternal injection of potassium caused tachycardia, slight rise of arterial pressure and rapid shallow breathing, and it was suggested that these changes may depend on medullary stimulation. One possibility, for which we have no supporting evidence, is that direct or reflex vasodilation account for the observed cardiovascular responses.

It is only possible to guess at the precise mechanism by which vasoconstriction with presumably relative local ischemia may cause central vasomotor inhibition. It is unlikely that it is due to change in carbon dioxide tension because an increase stimulates vasomotor centers and should result in rise of pressure, whereas warming of cerebrospinal fluid causes a decrease of carbon dioxide tension but is also associated with a stimulant effect.

It was considered that warming of cerebrospinal fluid might speed destruction of vasoconstrictor agents and thus nullify their central inhibitory effects. It is probable that any such action was an insignificant one since there was prompt return of the same degree of central inhibition when warming was discontinued. It is also likely that vasodilator drugs did not oppose the action of vasoconstrictor drugs by facilitating absorption into the systemic circulation, for their action was of short duration compared with the inhibitory action of 5HTP and was followed by return of the same degree of central inhibition.

Generalized hypothermia decreases systemic arterial pressure, heart rate and cerebral blood flow as well. Localized cerebral hypothermia has also been shown to cause lowering of arterial pressure and bradycardia and to cause decrease in cortical electric activity. The experiments reported here are in accord with these observations, although it is not known whether the common cardiovascular effects depend on the same final mechanism. Different areas of the brain concerned with vasomotor function apparently respond in different ways to changes in temperature. Newman et al. found a heat-sensitive region in the medulla that responded to rise in temperature by producing fall of arterial pressure. More generalized heating had the opposite effect in our experiments.

Marrazzi and Hart and Slocombe et al. found that norepinephrine and serotonin transiently inhibited central synaptic transmission when they were injected into a common carotid artery, and Trendelenburg has shown that histamine and serotonin facilitate transmission through the superior cervical ganglion. It is not known whether the change in local blood flow may have accounted in part for these results. Marrazzi and Hart do not think this is the case since the dosage given into the carotid artery was so small that systemic arterial pressure was not affected, and they add that the inhibitory effect of anoxia is much delayed compared with that of serotonin.

Ginzel showed that LSD or BOL given into the lateral ventricle blocked the carotid occlusion response and that serotonin given in the same manner did not influence the action of LSD. The present results, in which LSD and BOL did not modify the central inhibitory action of 5HTP in dogs, are consistent with
his observation. It is known that neither LSD nor BOI has any marked antagonism to the cardiovascular effects of serotonin in dogs. Since completion of these experiments there has appeared a publication by Bhargava and Tangri in which some experiments similar to ours are described. Serotonin (1 to 2 mg.) injected into a lateral ventricle was found to cause lowering of arterial pressure and inhibition of the carotid occlusion response, and it was concluded that these effects depended on central inhibition of sympathetic outflow. However, they were unable to demonstrate any central effect of norepinephrine (up to 1 mg.), vasopressin (2 to 4 units) or histamine (up to 750 µg.), and they concluded that local cerebral vascular changes were not responsible for the central effects of serotonin. Since dogs were employed and the technic was essentially the same, we have no explanation for the different results. We found norepinephrine to be the most powerful central inhibitory vasoconstrictor drug tested, and it had a marked effect in dosage much smaller than that employed by Bhargava and Tangri. Effective dosage of histamine in our experiments was also smaller than the maximum dosage employed by them.

Summary
Several vasoconstrictor drugs and reserpine administered into a cerebral lateral ventricle inhibited the reflex pressor response to occlusion of the common carotid arteries in both anesthetized and unanesthetized dogs. This effect was opposed by central administration of the vasodilator drugs, nitroprusside and histamine. Serotonin, norepinephrine and their precursors, 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine, and reserpine caused lowering of arterial pressure and slowing of heart rate as well as inhibition of the carotid occlusion response; angiotensin and vasopressin did not. The central inhibitory effect of norepinephrine was opposed by phentolamine; that of 5-hydroxytryptophan was not affected by lysergic acid diethylamide or its brom derivative.

Cooling of cerebrospinal fluid, which presumably caused local vasoconstriction, also caused inhibition of the carotid occlusion response, hypotension and bradycardia, and these effects were counteracted by central injection of vasodilator drugs. Warming of cerebrospinal fluid, presumably associated with local vasodilation, opposed the central inhibitory effect of vasoconstrictor drugs and of reserpine.

In view of the consistently opposite effects of vasoconstrictor and vasodilator drugs and procedures on central vasomotor sympathetic activity, it is tentatively concluded that these effects depend on change in local blood flow. The acute cardiovascular effects of reserpine of central origin are probably due to local decrease of tissue perfusion caused by serotonin, norepinephrine or other vasoconstrictor agent released from a bound to an active form.

Synopsis
Because of the consistently opposite effects of vasoconstrictor and vasodilator drugs and procedures on central vasomotor activity, it is concluded tentatively that these effects are due to change of local blood flow. The acute cardiovascular effects of reserpine of central origin probably depend on the same mechanism.

Summario in Interlingua
Plure drogas vasoconstrictori e reserpina administrate in unilateral ventriculo cerebral inhibiva le reflexa responsa pressori al occlusion del arterias carotidie communes tanto in anesthesiati como etiam in non anesthesiati canes. Iste effecto esseva contrariate per le administracion central del drogas vasodilatatori nitroprussida e histamina. Serotonina, nor-epinephrina, lor precursores 5-hydroxytryptophano e 3,4-dihydroxyphenylalanina, e reserpina causava re-ductions del tension arterial e rellentamientos del frequenti cardic e etiam le inhibition del responsa de occlusion carotidie. Angiotensina e vasopressiona non habeva isto effectos. Le effecto contro-inhibitori de norepinephrina esseva contrariate per phentolamina; illo de 5-hydroxytryptophano non esseva affiti-e per diethylamida de acido lysargie o su derivato bromic.

Frigidation del liquido cerebrospinal, que presumite-mente causava vasoconstriction local, causava etiam inhibition del responsa de occlusion carotidie, de hypotension, e de bradycardia; e iste effectos esseva contrariate per le injection central de drogas vasodilatatorti. Calentacion del liquido cerebrospinal, presu-
mitamente associato con vasodilatazione local, con-
trariamente a consequenze locali e di reserpina.

Viste le uniformemente opposte effetti di drogas 
vasoconstrictori e vasodilatatori da un lato e da 
manovre interessanti le attività sympatiche centro-
vasomotori del altere, la conclusione tentativa è for-
mulata che iste effetti dipendono da alterazioni in 
le flussi di sanguine locali. Le acuti effetti cardio-
vasculari di reserpina di origine centrali è probabile-
mente causato per un local riduzione del perfusione 
tissutale con effetto di serotonina, norepinefrina, o 
altri agenti vasoconstrictori liberati ab lor forma 
ligate a in un forma attiva.

Synopse.? Viste le uniformemente opposte effetti 
de drogas vasoconstrictori e vasodilatatori da un latere 
e di manovre interessanti le attività centro-vaso-
motori del altere, le conclusioni sono formulate ta-
tivamente che iste effetti è lo risultato di altera-
tioni nel flusso di sanguine locali. Le acuti effetti 
cerebrovascolare di reserpina di origine centrali è 
probabilmente dipendente dal medesimo meccanismo.

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