Effects of Intracarotid Injections of Hypertonic Solutions on Arterial Pressure in the Rabbit

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The intracarotid injection of hypertonic saline in the rabbit induces a brief drop in arterial pressure, followed by a somewhat longer pressor phase. The "osmoreceptors" activating these changes are related to the hindbrain and are not the elements responsible for releasing neurohypophysial hormones. The pressure changes are not prevented by vagotomy, denervation of the carotid body and sinus, adrenalectomy, or parasympathetic blockade. The blood pressure effects are interpreted as resulting from sequential inhibition and stimulation of medullary vasomotor centers by the hypertonic solutions.

Impulses from a variety of chemoreceptors and baroreceptors, impinging on the neuraxis, affect the central nervous control of arterial pressure. There is evidence of a hierarchy of interdependent levels of vasomotor control in the central nervous system which are primarily reflex in their mode of action. One important vasomotor center lies at the level of the medulla oblongata. In certain species an additional centrally controlled vasodilator system has been shown to exist.

The reflex discharge of the neurohypophysial hormone vasopressin or ADH has a questionable influence on arterial pressure. It has been shown that a sudden sustained fall in blood pressure such as occurs in severe hemorrhage will cause the release of this hormone from the posterior pituitary. Verney has demonstrated that elevation of the osmotic pressure of cerebral blood by injecting hypertonic saline into the carotid artery will activate hypothetical osmoreceptors in the presencephalon which cause ADH to be released. Recently the effects on the electroencephalogram (EEG) produced by injecting hypertonic solutions into the carotid artery were studied in this laboratory. This work permitted time relations of EEG changes, pituitary activation and hormone release to be established. In addition there were indications of sympathetic system activation as judged by the inhibition of the milk-ejection response, stimulation of uterine contractions and blood pressure changes.

In the present study the pressure changes in response to intracarotid injections of hypertonic solutions have been examined in detail. The results indicate that the solutions act directly on vasomotor elements lying in the lower pons or medulla oblongata, or both. They characteristically induce a biphasic change in arterial pressure—a depressor phase followed rapidly by a pressor effect. The biphasic response is mediated by vasomotor nerves to the blood vessels and does not involve to any appreciable degree either the neurohypophysis or the adrenal medulla.

Methods

New Zealand rabbits weighing from 2.2 to 5.3 Kg. were used. In 40 acute experiments the surgery was performed under ether anesthesia. The rabbits were later immobilized with d-tubocurarine and respiration was maintained with a pump. Eight additional experiments were conducted under sodium pentobarbital (30 mg./Kg.) without curare. In all animals the trachea was intubated and the left common carotid artery was threaded rostrally with a polyethylene tube. The carotid bodies and sinuses were denervated under a binocular dissecting microscope, either
INTRACAROTID INJECTIONS OF HYPERTONIC SOLUTIONS

TABLE 1.—Influence of Neurosurgical Procedures on End-Arterial Pressure Changes Induced by Rapid Injections of Intracarotid Hypertonic Saline (3 ml. 0.5 M NaCl)

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>Number of animals</th>
<th>Pressure changes (mm. Hg ± S. E.)</th>
<th>p Values for difference from unoperated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Drop</td>
<td>Rise</td>
</tr>
<tr>
<td>Unoperated</td>
<td>34</td>
<td>−23(±8)</td>
<td>+45(±4)</td>
</tr>
<tr>
<td>Carotid sinus and body</td>
<td>7</td>
<td>−19(±3)</td>
<td>+39(±15) 0.6 0.7</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>7</td>
<td>−12(±5)</td>
<td>+26(±6)  0.2 0.0*</td>
</tr>
<tr>
<td>Brainsstem transection</td>
<td>14</td>
<td>−24(±4)</td>
<td>+39(±7)  0.9 0.4</td>
</tr>
</tbody>
</table>

*The only operative procedure that approaches significance with respect to alteration of the saline-induced pressure pattern is vagotomy, after which the rise is more definitely affected than the drop.

RESULTS

A typical example of the changes in arterial pressure following intracarotid injections of hypertonic saline solutions is seen in figure 1B. Characteristically, the pressure dropped some 20 mm. Hg within a very few seconds and returned to its preinjection level within 10 sec. Instead of remaining there it continued upward some 40 to 50 mm. Hg, reaching a peak at about 15 sec. post-injection from which it slowly (15 or more sec.) returned to its preinjection level. Neither the intracarotid injection of isotonic saline (fig. 1A) nor the intravenous injection of hypertonic saline (fig. 1C) produced the response. This biphasic change in arterial pressure following intracarotid injections of hypertonic saline was observed in 46 of 48 rabbits. Thirty-four of these animals received 3 ml. 0.5 M NaCl and the results are included in table 1 for comparison with results after neurosurgical procedures.

During the initial phase of lowering of the arterial pressure in response to hypertonic saline, the heart rate usually slowed as seen in the electrocardiogram (fig. 2B). This observation led to the hypothesis that the phase of depression might be attributable entirely to cardiac inhibition of vagal origin. However, bilateral vagotomy, while eliminating the bradycardia, did not entirely counteract the drop in arterial pressure nor the subsequent pressor phase (fig. 2C and table 1). Part of the initial rapid fall in pressure frequently observed prior to vagotomy (figs. 2B, D) is attributable to centrally-activated bradycardia in that the marked heart slowing on

The blood pressure record is not seen after vagotomy. A brief period of delayed cardiac arrhythmia was often seen in vagotomized rabbits, but it was not related to either of the pressure changes (fig. 2C).

On the afferent side of the reflex following intracarotid injections, the carotid body and carotid sinus were suspect. However, the biphasic response to hypertonic saline was retained in 7 rabbits in which these receptors were deafferented surgically (table 1 and fig. 2D). In an additional 9 animals the response persisted through pentobarbital anesthesia of sufficient depth to eliminate any painful effect of the injection. The mean pressure changes in this group were $-20 \pm 4 +19 \pm 4$ mm Hg, the pressure rise being significantly less than in the curarized preparations. Under deep surgical anesthesia the pressor responses were markedly diminished.

That the alterations in arterial pressure were not an exclusive effect of excess NaCl per se was evidenced by the finding that hypertonic glucose would also induce a biphasic response in arterial pressure. However, somewhat higher concentrations of glucose were needed to produce an equivalent response, a finding consistent with that of Verney. These observations led to the tentative conclusion that the effective stimulus was the elevation of the osmotic pressure of the blood acting directly on the brain. Hypertonic urea was employed as a control because of its known ability to penetrate into osmosensitive receptors, but results with this substance were highly variable. Among 10 animals receiving hypertonic urea, 3 failed to produce a change in arterial pressure, 4 had a drop in pressure and 3 showed a biphasic response. This general variability held whether concentrations of 1 or 2.4 M were used.

Most of the experiments reported here were conducted in conjunction with a study of EEG activity in various parts of the forebrain (fig. 3A). Certain EEG channels show the triphasic EEG response to hypertonic saline and glucose described by Sawyer and Gernandt. There is an apparent parallelism between the degree of alteration of the EEG and the extent of the biphasic response in arterial pressure. Whereas the two phenomena will later be shown to be independent, this parallelism suggests a similarity in functional characteristics of the independent receptors.

In a series of 14 animals, branches of the carotid trunk were occluded to determine the general area of the brain responsible for the blood pressure response. With the external carotid clamped and either the occipital or internal carotid artery occluded the response was still present. The occipital artery has been seen to anastomose with the vertebral-basilar system of vessels, as does also the internal carotid more rostrally at the circle of Willis. The solutions can thus reach the brain through either of these latter routes, but with the occipital tied and the internal carotid clamped, the blood pressure response was practically eliminated. Removal of the clamp
from the internal carotid then restored the response. At this stage of the evidence it would be impossible to deny either that prosencephalic "osmoreceptors"1 activating EEG changes and neurohypophysial function might induce the arterial pressure changes or that the EEG changes might result secondarily from the changes in arterial pressure.

In an effort to analyze priorities in these functional changes and to localize further the regions in the central nervous system controlling the blood pressure changes described, the brainstem and spinal cord were severed at various levels. After recovery from the shock of the surgery the biphasic response in arterial pressure was observed to have survived transection of the brainstem between the rostral midbrain and the upper pons in 12 of 14 rabbits (figs. 3B, 4C). In figure 3B it is clear that after severance of the brainstem at the level of the pons, neither the biphasic response in arterial pressure nor the charac-
The data in figure 5 further divorce any causal relationship between the changes in arterial pressure and EEG alterations in response to hypertonic solutions. In figure 5A, a volume of hypertonic saline too small (0.3 ml.) to alter the EEG had a positive effect on the arterial pressure. In the same rabbit after severing the spinal cord at T6 it was possible, with an increased volume of saline (1.0 ml.), to induce EEG changes in the virtual absence of blood pressure changes, showing definitely that the EEG alterations are not causally dependent upon systemic changes in arterial pressure.

The possibility that adrenal medullary hormones might play a major role in the alterations of arterial pressure was eliminated by demonstrating a retention of the effects after bilateral adrenalectomy (fig. 6B) in 3 animals. The likelihood that the early depressor phase might be mediated by cholinergic vasodilator fibers was investigated by administering atropine sulfate to both adrenalectomized and nonadrenalectomized animals. In a total of 7 animals no selective effect on either phase was observed (fig. 6C), though the absence of bradycardia revealed that peripheral parasympathetic blockade had been achieved by 3 mg./Kg. atropine sulfate. Dosages up to 15 mg./Kg. (fig. 6D) tended to reduce somewhat the magnitude of both phases, perhaps due to blockade of central or preganglionic synaptic transmission, or both.

Although earlier work in this laboratory has shown that intracarotid injections of hypertonic saline or glucose of the order of magnitude employed here cause the release of neurohypophysial hormones in the rabbit, no evidence could be found that vasopressin contributed to the pressor response. Dosages of Pitressin in a presumably physiologic range (10 mU.) produced no detectable effect on blood pressure; and large doses (100 mU. or more) gave only mild pressor changes. When the release of neurohypophysial hormones is detected by the milk-ejection technic of Cross and Harris and compared temporally with the blood pressure changes (fig. 7) it is seen...
that the biphasic response in arterial pressure is largely completed several seconds before the surge of released hormones causes ejection of milk. Thus, the latency of the release of the hormone following intracarotid injections also makes it unlikely that vasopressin is involved in the response.

**DISCUSSION**

By surgical and pharmacologic intervention and simple calculations based on known latencies it has been possible to exclude certain structures and systems from consideration as factors controlling the changes in arterial pressure resulting from intracarotid injections of hypertonic solutions.

Since the pressure changes are retained after transection of the brainstem, a vasomotor system involving Verney's proencephalic "osmoreceptors" would require a hu-
The changes in EEG and arterial pressure, evoked by intracarotid injection of hypertonic saline, are largely completed before the effects of released endogenous neurohypophysial hormone are recorded (milk ejection). The latency for milk ejection following intravenous injection of exogenous vasopressin or oxytocin is approximately 70 sec.

The depressor phase might at first appear to be a simple baroreceptor reflex. This possibility was excluded by: (1) failure to evoke the response with isotonic saline injected at the same volume and rate as the hypertonic injections, (2) retention of the response after deafferentation of the carotid sinus, and (3) retention of the response in the absence of bradycardia after vagotomy. These observations and the time factors of the response, which rule out its production from receptors located elsewhere than in the carotid bed appear to exclude the Bezold-Jarisch reflexes.14

The failure of denervation of the carotid body and sinus to affect either phase of the arterial pressure response indicates that the osmoreceptors responsible for blood pressure changes are located outside these structures. Verney also failed to find osmoreceptors in this region.8

The maintenance of the blood pressure response in the absence of the adrenals is consistent with the findings of Folkow1 that vascular smooth muscle effectors are controlled primarily by vasomotor influences rather than adrenal epinephrine. This author found that even in extreme anoxia the neurogenic effect on blood vessels preceded and overshadowed that of the adrenal catechols.

Sympathetic cholinergic vasodilator fibers have been described in certain species5 but have not been demonstrated in the rabbit.15,16 Our results with atropine agree with the latter reports. The more likely explanation for the depressor phase obtained in our experiments is that a temporary suppression of vasomotor tone is produced by direct inhibition of medullary vasomotor centers. The relative independence of these centers in responding to hypertonic solutions is consistent with their general independence from higher centers in the maintenance of arterial pressure.1

Circuit B of figure 8 illustrates the pathways which the present data support for control of the arterial pressure changes described. Inasmuch as the vagus and any peripheral cholinergic vasodilator system have been virtually eliminated as possible efferent mechanisms, the initial depressor effect would appear to be transient inhibition of the vasomotor center followed quickly by stimulation...
of the center. Both effects would be transmitted to peripheral arterioles by the sympathetic outflow from the spinal cord. Transection of the cord at C1 would eliminate all and at T6 a large part, of this outflow.

Circuit \( A \) in figure 8 has different functions and it has received more attention than circuit \( B \). Other recent studies have shown that hypertonic saline in amounts similar to those employed here activates the release of neurohypophysial hormones in the rabbit as evidenced by the milk-ejection technique. In the course of the activation, characteristic EEG responses have been recorded, especially in the region of the olfactory tubercle, suggesting that this may be the site of Verney’s osmoreceptors. The EEG changes are only incidental in this study, but they offer a general means of telling whether the amount of injected solution is adequate to activate osmoreceptive elements. Future work will attempt to record electrical changes in the medulla under conditions in which the vasomotor center is known to be activated.

**SUMMARY**

The intracarotid injections of hypertonic saline or glucose solutions induced a biphasic effect on arterial pressure consisting of a brief depressor effect, followed by a somewhat longer pressor component. Hypertonic urea solutions gave variable results. The biphasic arterial pressure changes persisted after bilateral vagotomy and deafferentation of the carotid sinus and carotid body. Bradycardia of vagal origin may contribute to the initial part of the depressor phase.

Transections of the brainstem from the mesencephalo-diencephalic junction to the midpontine level fail to affect the biphasic response. Spinal cord section at the sixth thoracic level considerably curtails the response, and transection at the first cervical level eliminates it completely. Neither adrenal medullary hormones nor peripheral cholinergic vasodilators could be implicated in the response since both phases survive adrenalectomy and parasympathetic blockade with anticholinergic drugs.

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**SUMMARIO IN INTERLINGUA**

Injectiones intracarotidic de solutiones hypertonic de sal o glucosa produciveva in conilios un effecto biphasic in le tension arterial. Le prime phase esseva un breve effecto depres-
sori. Esto eseva sequite per un aliquie plus longe componente pressori. Solutiones hypertonic de urea produceva effectos variabile. Le alterationes biphasis del tension arterial persisteva post vagotomia bilateral e disafferen
tation del sinu e del corpore carotidic. Bradycardia de origine vagal contribuie possibilemente al parte initial del phase depressori.

Transsectiones del caudice cerebral ab le junction mesencephalo-diencephalic usque al nivello medio-pontiu non affice le responsa bi-
phasic. Le section del cordon spinal al nivello del sexte vertebra thoracic abbrevia le responsa considerabilmente, e le section del cordon spinal al nivello del prime vertebra cervical elimina le completolemente. Ni hormones adrenomedullar ni vasodilatatores cholinergic peripheric poteva esser incriminate como factores causative del resposta, proque le duo phases mentionate supervive a adrenalecostomia e a bloco parasympathic efectuate per medio de drogas anticholinergic.

Le liberation de hormones neurohypophysie in responsa a stimulation osmoreceptori occurre troppo tarde e in quantitates troppo miere pro activar le prime o mesmo le secunde del duo phases.

Il pare que le solutiones hypertonic exercer le effects in le tension arterial tanto per influentias inhibitori como etiam per influentias stimulatori in le centros vasmotorni del medul-
la. Le resultante alterationes de tension es, per consequente, effectuate per alterationes phasic in le tono sympathic.

Concomitate alterationes prosencephalic del electroencephalogramma non relationate a iste systema, es implicate in mechanismos de reflexo per que solutions hypertonic stimula le liberation de hormones neurohypophysie via circuitos prosencephalic e diencephalic.

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