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

By ROBERT C. HOLLAND, PH.D., JOHN W. SUNDSTEN, A.B., AND CHARLES H. SAWYER, PH.D.

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.

Manipulations of cerebral blood pressure, including changes in blood flow, are mediated by vasomotor centers in the brain. These centers are located in the medulla oblongata and are influenced by a variety of factors, including chemoreceptors and baroreceptors. 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 presencephalanx, 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
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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>Unoperated</td>
<td>34</td>
<td>Drop: -23(±8) Rise: +45(±4)</td>
<td></td>
</tr>
<tr>
<td>Carotid sinus and body</td>
<td>7</td>
<td>Drop: -19(±3) Rise: +39(±15)</td>
<td>0.6 0.7</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>7</td>
<td>Drop: -12(±5) Rise: +26(±6)</td>
<td>0.2 0.0*</td>
</tr>
<tr>
<td>Brainstem transection</td>
<td>14</td>
<td>Drop: -24(±4) Rise: +39(±7)</td>
<td>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...
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A PRE-LESION

B POST-LESION

FIG. 3. Failure of transection of the brainstem at the midpontine level to eliminate changes in arterial pressure or EEG following intracarotid injections of hypertonic saline. These and subsequent EEG tracings: ADT, anterior dorsal nucleus of thalamus; AMYG, amygdala; CC, corpus callosum; DB, diagonal band; DMT, dorsomedial nucleus of thalamus; EKG, electrocardiogram; IC, insular cortex; LHA, lateral hypothalamic area; MPO, medial preoptic area; MTH, mammillothalamic tract; N.Acc, nucleus accumbens; OC, optic chiasma; OLF.TUB, olfactory tubercle; OT, optic tract; SEPT., septum; SM, stria medullaris; SMA, supramammillary area; VMT, ventromedial nucleus of thalamus.

from the internal carotid then restored the response. At this stage of the evidence it would be impossible to deny either that pre-encephalic "osmoreceptors" 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-
FIG. 4. Arterial pressure and electrocardiographic tracings in an unusual animal in which a rapid pressor phase (A) was evoked by injecting usually innocuous isotonic saline. This early rise in pressure masked the depressor phase ordinarily obtained with hypertonic saline (B). The rapid pressor response was controlled by a rostral vasomotor center, since after midbrain transection the typical biphasic response was observed (C). Further effects of sectioning the spinal cord at T6 and C1 are seen in D and E. Times, sequence between surgery and intracarotid injection.

teristic EEG changes have been lost. Thus, the EEG changes are at least largely independent of influences from the caudal brainstem and the arterial pressure changes are similarly independent of nervous projections from more rostral areas.

The animal in figure 4 showed an interesting rapid pressor phase, perhaps of emotional derivation, which masked the initial depressor phase (fig. 4B) until this component of prosencephalic origin was eliminated by transecting the midbrain (fig. 4C). Severeing the spinal cord at the sixth thoracic level (fig. 4D) greatly reduced the response, presumably by removing the massive sympathetic outflow below this level. Transection of the cord at the first cervical level abolished the response (fig. 4E).

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 prosencephalic “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.

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 technic. 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 midponsile 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.

The release of neurohypophysial hormones in response to osmoreceptor stimulation occurs too late and in insufficient quantity to activate either of the two phases.

The hypertonic solutions appear to exert their effects on arterial pressure by both inhibitory and stimulatory influences on medullary vasomotor centers. The resultant pressure changes are thus brought about by phasic alterations in sympathetic tone.

Concurrent procencephalic EEG changes, unrelated to this system, are involved in reflex mechanisms by which hypertonic solutions stimulate the release of neurohypophysial hormones via prosencephalic and diencephalic pathways.

**Acknowledgments**

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**Sommario in Interlingua**

Injestiones intracarotidide de solutiones hypertonic de sal o glucosa producivea in conillios un efecto biphasic in le tension arterial. Le prime phase esseva un breve efecto depres-
sori. Isto esseva sequite per un aliique plus longe componente pressori. Solutiones hyper-tonic de urea produceva effectos variabile. Le alterationes biphasic del tension arterial persisteva post vagotomia bilateral e disafferen-tation del sinus e del corpore carotidic. Brady-cardia de origine vagal contribue 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 considerablemente, e le section del cordon spinal al nivello del prime vertebra cervical elimina lo completamente. Ni hormones ad-rene-medullar ni vasodilatatores cholinergic peripheric poteva esser incriminate como fac-tores causativo del respansa, proque le duo phases mentionate supervive a adrenalectomia e a bloco parasympathic effectuate per medio de drogas anticholinergic.

Le liberation de hormones neurohypophysee in responsa a stimulation osmoreceptori oc-curre troppo tarde e in quantitates troppo micre pro activar le prime o mesmo le secunde del duo phases.

Il pare que le solutiones hypertonc exerce lor effectos in le tension arterial tanto per influenzaes inhibitori como etiam per influentias stimulatori in le centros vasmotori del medul-la. Le resultante alteraciones de tension es, per consequente, effectuate per alterationes phasic in le tono sympathic.

Concomitante alterationes prosencephalic del electroencephalogramma, non relationate a iste systema, es implicate in mechanismos de reflexo per que solutiones hypertonc stimula le liberation de hormones neurohypophysee via circuitos prosencephalic e diencephalic.

REFERENCES
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