The Inductive Action of Alcohol-Killed Chick Hensen's Node on Amphibian Ectoderm[1]

Recent studies have shown that Hensen's node of avian blastoderm exerts an inductive influence on competent avian ectoderm, even when killed by alcohol or heat. In the experiments of LEIKOLA and MCCALLION[2], in which the ectoderm was cultured in vitro for 24–28 h, the killed Hensen's node induced neural structures with a relatively high frequency. In subsequent experiments by VISWANATH et al.[3], the ectoderm was cultured for 10–12 days in chick coelomic cavity, and both endodermal and neural structures were induced, although with lesser frequency. Since Hensen's node is in many respects comparable, if not homologous, to the amphibian upper blastoporal lip, it has appeared to us of interest to apply the killed Hensen's node on competent amphibian ectoderm, which is known to be readily induced by different dead inductors, including the killed blastoporal lip.

Hensen's nodes were excised from blastoderms of White Leghorn eggs incubated at 38 °C for 16–18 h (Hamburger-Hamilton stage 4). The nodes were killed by immersing them in 70% alcohol for some minutes, whereafter they were rinsed thoroughly with Holtfreter-Ringer solution. The competent ectoderm was obtained from young *Triturus vulgaris* gastrulae. 'Sandwiches' were made with the killed node between 2 pieces of ectoderm, and these explants were cultured for 7–10 days at 18 °C in Holtfreter-Ringer solution. They were fixed in Bouin's fluid, sectioned, stained with Borax-Carmine and Picroblueblack and examined histologically.

Of a total of 25 explants, 18 survived. In all of them archencephalic structures, including forebrain parts and, more frequently, eyes were found. In addition to them, only undifferentiated epidermis and, in some cases, more regular epidermis had been formed. Hindbrain, spinal cord or mesodermal structures were never present.

This result indicates that the alcohol-killed avian Hensen's node acts as a typical neuralizing inductor in amphibian ectoderm. Its action is thus comparable with that of various other heterogenous inductors, such as liver or different other heat-treated tissues, which contain only a neuralizing inductive agent and thus cause only archencephalic inductions[4]. On the other hand, the action of a killed Hensen's node on competent avian ectoderm seems to be more varied[3]. Thus it seems reasonable to conclude that the undetermined avian and amphibian ectoderm does not react in a similar way if the same inductor is used as a primary trigger. This conclusion is well in accordance with the results of ROSTEDT[5,6], who has applied different heterogenous inductors on avian blastoderm and obtained inductions that are remarkably different from those obtained earlier in amphibian ectoderm with the same heterogenous inductors.

Résumé. Le nœud de Hensen du blastoderme du Poulet a été tué par l'alcool et appliqué comme inducteur sur l'ectoderme des gastrules de *Triturus vulgaris*. Seules des structures archencéphaliques ont été induites, ce qui indique que l'ectoderme des Amphibiens réagit au même inducteur d'une manière différente de celui du Poulet.

J. R. VISWANATH, A. LEIKOLA and S. TOIVONEN

Laboratory of Experimental Embryology, Department of Zoology, University of Helsinki (Finland), 14 August 1968.

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Responses of Resistance and Capacitance Vessels at Various Frequency Electrical Stimulation of Sympathetic Nerves

It is known that maximal reactions of resistance and capacitance vessels are induced by various frequencies of sympathetic stimulation. One group of authors[1] observed maximal response of cat's hindlimb resistance vessels at 16 cps and greatest reactions of capacitance vessels in the same preparation at 6 cps. The other group of authors[2], in experiments on dogs, noted the same value of changes at 10–20 cps for resistance vessels and at 10 cps for capacitance vessels. It was also shown[3] that maximal changes of capacitance vessels in the skin of hindlimbs arose at 15–20 cps. It should be stressed, however, that the results noted above were observed in experiments on hindlimb preparation, and it was not quite clear whether the parameters were of equal value for all vascular zones or whether the induction of maximal reactions in each of them needed particular frequencies. The purpose of this work was to elucidate the optimal frequency parameters for inducing maximal reactions of resistance and capacitance vessels of brain and lungs, as compared to reactions of resistance and capacitance vessels situated below the abdominal aorta bifurcation.

Technique. Experiments were performed on cats (49) anaesthetized with urethane (1 g/kg). Reactions of resistance vessels were identified by the changes of perfusion pressure and reactions of capacitance vessels by the value of maximal output or storing of blood. A vascular zone under investigation was perfused by means of a constant blood flow pump. Venous outflow from this zone was directed into the measuring cylinder from which the blood

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was returned into the venous system of the animal by the second canal of perfusion pump[4,5]. The efficiency of both perfusion pumps being constant, changes of pressure in the arterial part of the vascular zone reflected reactions of resistance vessels, while changes of the venous outflow reflected the reactions of capacitance vessels.

When reactions of cerebral vessels were studied, the influence of extracranial vessels and systemic arterial pressure was precluded. The blood from the brain was flowing out through v.v. jugularis and through the artificially made hole in the transversal sinus, other ways of blood outflow being closed. Cervical sympathetic chains were divided, their cranial ends being stimulated below the superior cervical sympathetic ganglions.

The reactions of the pulmonary vessels were studied on cats with the thorax opened under artificial breathing. Vascular reactions were observed after the electrical stimulation of the left stellate ganglion. Reactions of vessels situated below the abdominal aorta bifurcation were studied after simultaneous stimulation of peripheral ends of cut sympathetic chains on the level of the 5–6 lumbar segments.

Electrical nerve stimulation was performed with a rectangular wave stimulator. The parameters of stimulation were the following: voltage 3–15 V; impulse duration, 5 msec; frequencies varying from 3–50 cps.

Results. Stimulation of cervical sympathetic nerves was followed in all 16 experiments by an increase of perfusion pressure in cerebral vessels. The magnitude of these reactions rose as the frequency of impulses was increased from 3–30 imp./sec (Figure 1, r). Further rise of impulse frequency was accompanied by a decrease of reaction magnitude shown by cerebral resistance vessels. The increase of blood output from the venous part of cerebral vascular bed after stimulation of cervical sympathetic chains was maximal at frequency of 10 cps and decreased when a further increase of impulse frequency up to 50 cps was caused (Figure 1, c). In 42% of experiments the increased blood output was followed by a storing of blood with average duration of 7.7 min. Differences related to the course of reaction between resistance and capacitance vessels were noted, too. Reactions of cerebral resistance vessels had response latency of 1.6 $\pm$ 0.2 sec and reactions of cerebral capacitance vessels 3.7 $\pm$ 0.3 sec. The reactions of resistance vessels reached their highest point after 28.0 $\pm$ 1.3 sec; in contrast, venous output reached its maximum only after 19.0 $\pm$ 1.8 sec.

As a result of the left stellate ganglion stimulation (8 animals), the increase of perfusion pressure in vessels of the left lower lung lobe was observed in 77 cases (81%), the perfusion pressure decreased in 5 instances (53%) and showed no changes in 13 (13.7%). The maximal perfusion pressure increase was observed at the 50 cps stimulation (Figure 2, r); however in all experiments maximal value of perfusion pressure increase was not higher than 3.1 mm Hg. Venous outflow from the same pulmonary lobe decreased in 79 instances (96.4%) and was unchanged in 3. The maximal value of the storing of blood in pulmonary vessels was observed at 40 cps frequency, while a decrease or increase of frequencies caused capacitance vessel reactions of less magnitude (Figure 2, c).

Response latency for pulmonary resistance vessels was equal to 3.4 $\pm$ 0.3 sec, of capacitance to 1.3 $\pm$ 0.2 sec.

Reactions of resistance vessels situated below the abdominal aorta bifurcation in response to the sympath-

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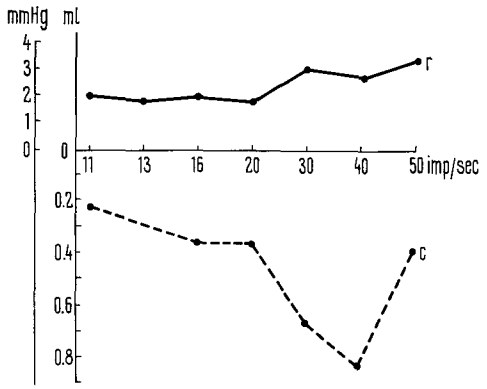

Fig. 2. Reactions of resistance (r) and capacitance (c) vessels of the lower pulmonary lobe at various frequency of stimulation of the left stellate ganglion (values averaged from 8 experiments). On the abscissa, imp./sec; on the ordinate, above the abscissa, changes of perfusion pressure, under the abscissa, values of decreasing of blood outflow from the pulmonary veins.

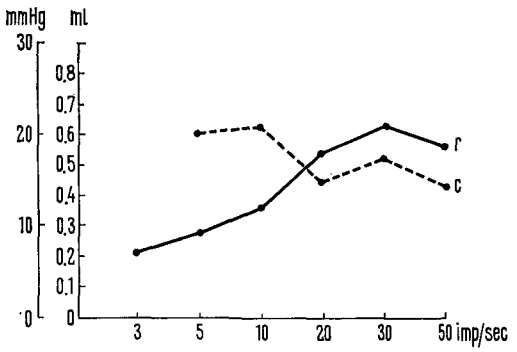

Fig. 1. Reactions of resistance (r) and capacitance (c) cerebral vessels at the different frequencies of electrical stimulation of cranial ends of cervical sympathetic chains (values averaged from 16 experiments). On the abscissa, imp./sec; on the ordinate, scale in mm Hg, changes of perfusion pressure, scale in ml, values of venous blood output.

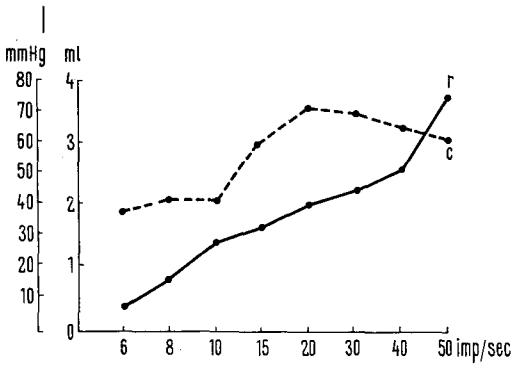

Fig. 3. Reactions of resistance (r) and capacitance (c) vessels, situated below the abdominal aorta bifurcation at the various frequencies of stimulation of lumbar sympathetic chain (values averaged from 25 experiments). Designations same as in Figure 1.

etic stimulation were constrictive. In all experiments (25), the greater the stimulation frequency (in range from 6–50 cps), the greater the magnitude of resistance vessel reactions in this vascular zone (Figure 3, r). Capacitance vessel reactions were observed through the whole range of frequencies used. The maximal value of these vessel reactions took place at the frequency of 20 cps, further increase of frequency led to a lesser magnitude of vascular reactions (Figure 3, c). Response latency for resistance vessels amounted to 2.2 ± 0.5 sec and 5.0 ± 0.6 sec for capacitance.

The great individual variability of resistance and capacitance vessel reactions in different animal subjects at the same parameters of electrical stimulation should be noted, too. Thus under stimulation of lumbar sympathetic nerves with the frequency of 15 cps, the perfusion pressure increased by 6 mm Hg with one animal and by 66 mm Hg with another, venous output at the same frequency was 1.4 ml in one case and 5.6 ml in the other.

Results of experiments showed that there is a great difference between frequencies of stimulation, inducing maximal reactions of resistance and capacitance vessels for various vascular zones.

Conclusion. (1) The maximal reactions of cerebral resistance vessels arise at the stimulation frequency of 30 cps, the maximal reactions of capacitance vessels at the frequency of 10 cps. (2) Reactions of resistance vessels in the pulmonary lobe increase in the range of stimulation frequencies from 11–50 cps, maximal reactions of pulmonary capacitance vessels are observed at the frequency of 30–40 cps. (3) Reactions of resistance vessels situated below the abdominal aorta bifurcation increase progressively in the range of frequencies from 6–50 cps, the magnitude of capacitance vessel reaction reaching its maximum value at 20 cps.

Выводы. Максимальные реакции резистивных сосудов мозга возникают при частоте стимуляции 30 имп/сек, емкостных - 10 имп/сек. Реакции резистивных сосудов легкого и расположенных ниже бифуркации брюшной аорты увеличиваются при нарастании частоты стимуляции до 50 имп/сек. Максимальные реакции емкостных сосудов легкого наблюдались при частоте 30-40 имп/сек, сосудов расположенных ниже бифуркации аорты при 20 имп/сек.

B. I. TKACHENKO, V. G. KRASILNIKOV,
S. A. POLENOV and G. V. CHERNJAVSKAJA

Institute of Experimental Medicine, Academy of Medical Sciences, Leningrad (USSR),
6 August 1968.

Influence of some Thiamine Antagonists on Frog Taste Receptors

According to VON MURALT and ZOTTERMAN[1], a crude extract of carp intestine containing a thiaminase, strongly inhibits the response of taste receptors to chemical stimuli when applied to the surface of the frog tongue. As a result thiamine (T) has been held to be directly involved in receptor activity.

In order to obtain more conclusive evidence for this hypothesis, we have investigated the influence on taste receptors of other antagonists of T like pyrithiamine (PT) and oxythiamine (OT).

The advantage of PT and OT over thiaminase preparations is that the former are pure compounds whilst the latter may contain several interferring substances.

In a first set of experiments carried out on isolated frog tongue preparations, PT, either applied to the tongue surface or infused intra-arterially, produced only a slight and transient decrease of receptor response to chemical stimuli[2]. However, the isolated frog tongue is not an appropriate preparation for testing the action of competitive inhibitors like PT and OT, because normal excitability of the taste receptors is maintained only for 15–20 min after interruption of the blood supply.

Better experimental conditions were obtained with frog tongue preparations intra-arterially perfused with oxygenated tyrode solution.

Under these experimental conditions, the receptors maintain their response to standard chemical stimuli unchanged over a period of several hours. Furthermore, by this technique it is possible to add the thiamine antagonists in known concentrations to the perfusion medium and to assure their intimate contact with the receptors for the desired length of time.

All the frog's tongue preparations were perfused through the lingual artery for 30 min with amphibian tyrode solutions, so that they might achieve equilibrium under the new conditions.

After 30 min either PT or OT was added to the perfusing medium. Receptor response was tested every 15 min by applying a solution of $CaCl_2$ containing $0.75 \times 10^{-4} M$/l Ca^{++} to the surface of the tongue for 30 sec.

The action potentials were picked up from one of the glossopharyngeal nerves with a suitable pipette, monitored on a Tektronix cathode-ray oscilloscope and counted on an electronic scaler. The perfusion technique and the recording assembly used in these experiments have been described elsewhere[3,4].

Four groups of experiments were carried out to assay the effect of PT on taste receptors. The first group of tongue preparations was perfused with plain tyrode solution. In the other 3 groups, PT, T and PT + T respectively were added to the perfusing medium. The concentration of each active compound in the medium was always $1.2 \times 10^{-4} M$.

Three separate groups of experiments were carried out to study the effect of OT on frog tongue preparations. In 1 set of experiments OT was perfused at a concentration of $2.6 \times 10^{-4} M$, in the other the concentration was higher, $5.3 \times 10^{-4} M$. The OT response was compared with a separate group of control preparations. For each treatment 8 experiments were carried out.

In Figure 1 the activity of the taste receptors, recorded during perfusion in our experiments, is reported as a percentage of the average receptor response obtained for

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