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3 June 1969

Afferent Discharges from Osmoreceptors in the Liver of the Guinea Pig

Abstract. *Afferent activtiy from vagal nerve filament of guinea pig liver was recorded. Perfusion of liver by hypertonic solution caused an increase in firing rate. There may be osmoreceptors in the liver.*

Osmoregulation is generally explained by a feedback loop between the hypothalamo-pituitary system and the kidney. The existence of osmoreceptors in the superior nucleus was established (*1*) in 1947. Haberich *et al.* (*2*) concluded that there are osmoreceptors in the liver because the volume of the urine changes after hyper- or hypotonic solutions are injected into the portal venous system. I now present neurophysiological evidence suggesting that there are osmoreceptors in the liver.

Experiments were performed on the livers of 60 guinea pigs. The animals were anesthetized with urethane, and the liver with the hepatic branch of the vagal nerve was excised from the body and perfused with standard Ringer solution or test solutions through a catheter inserted into the portal vein. The solution entered through the portal vein, circulated in the liver, and exited through the hepatic vein. The solution was saturated with 95 percent oxygen and 5 percent carbon dioxide and maintained at about 30°C. The perfusion pressure was about 50 cm-H_2O, and the perfusion rate was about 50 ml/min. An electrometer recorded the afferent impulse discharges from a fine filament of a nerve dissected from the vagal nerve branch of the liver; the records were preserved on magnetic tape. The activity of single or several nerve fibers was converted into standard pulses by a discriminator and analyzed by a digital computer. When a small filament was placed on recording electrodes, spontaneous afferent discharges were usually observed.

In the first experiment, hypertonic Ringer solution which contained 165 m*M* NaCl and had a total osmolarity of 346 milliosmole/liter was used as a test solution. As the range of osmolarity in mammalian systemic blood is supposedly ± 10 milliosmole, the osmolarity of this test solution must equal the maximum possible value expected in the portal venous blood after the absorption of the salt from the intestine. When the system was switched from standard Ringer solution to a test solution, an increase in the discharge rate was observed (Fig. 1). Thus, an increase in NaCl content in Ringer solution causes an increase in the discharge rate of vagal afferents from the liver.

In the next experiment, the relation between the afferent discharge rate and the concentration of NaCl in the test solutions was observed. Four different concentrations of Ringer solution which contained different amounts of NaCl were used. The higher the NaCl concentration, the higher the discharge rate (Fig. 2). Because the increase of 20 milliosmoles in the perfusion solution caused an increase in discharge rate, test solutions which differed by only 17 milliosmoles from

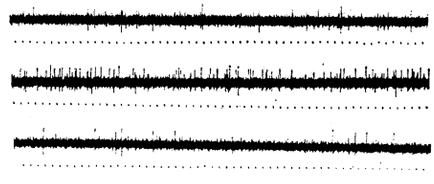

Fig. 1 (above). Afferent impulse discharges recorded from hepatic vagal nerve filament. (Top) Perfusion by standard Ringer solution (326 milliosmole/liter). (Middle) After change to hypertonic Ringer solution (346 milliosmole/liter). (Bottom) After changing back to standard Ringer solution (326 milliosmole/liter). Time mark: 0.1 second.

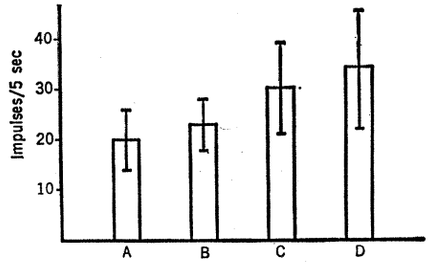

Fig. 2 (right). Relation between afferent discharge rate and concentration of perfusion solution.

	Concentration		Impulses/5sec.	
	NaCl (mM)	Osmolarity (mOsm)	Mean S.D. N=10	
A	150.2	316	20 ± 6	A<C (0.05>P>0.01)
B	155.2	326	23 ± 5	B<D (0.05>P>0.01)
C	160.2	336	30 ± 9	A<D (0.01>P)
D	165.2	346	34 ± 11	

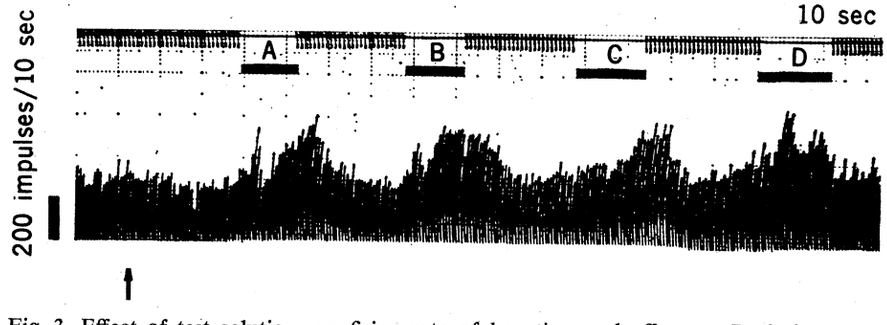

Fig. 3. Effect of test solutions on firing rate of hepatic vagal afferents. Perfusion pressure was increased by 20 cm-H_2O at the time indicated by the arrow. Horizontal bars indicate the time of perfusion by test solution. (A) Hypertonic mannose Ringer solution. (B) Hypertonic NaCl Ringer solution. (C) Hypertonic glucose Ringer solution. (D) Hypertonic sucrose Ringer solution.

standard Ringer solution were used in the third experiment. They were hypertonic Ringer solutions (343 milliosmole/liter) which were made by the addition of 17 m*M* of mannose, glucose, sucrose, or NaCl to standard Ringer solution. These test solutions also yielded an increase in the discharge rate (Fig. 3). When the perfusion pressure was suddenly increased by 20 cm-H_2O (Fig. 3), no change in the discharge rate was observed. I conclude that increase in discharge rate is in accord with increase in osmotic pressure in the perfusion solutions.

Sometimes a decrease in osmotic pressure, as well as an increase, caused an increase in discharge rate in multifiber preparations. Jacobs and Adachi (*3*) report osmoreceptors in the liver of the rat which are activated by hypotonic solutions. The same type of receptors thus occur in the guinea pig liver as well.

I suggest that osmoreceptors in the liver monitor osmotic pressure of the portal venous blood. They are linked to the central nervous system through the hepatic branch of the vagal nerve. These receptors may represent the afferent limb of reflex systems involved in the control of body fluid.

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Leukemia-Associated Antigens in the Mixed Leukocyte Culture Test

Abstract. *Patients with acute leukemia (blast cells in the peripheral blood) manifest antigens in the one-way mixed leukocyte culture test. Leukocytes from normal patients and leukocytes of patients in remission do not clearly show these antigens. These antigens could be leukemia-associated antigens in man.*

Tumor-associated transplantation antigens—antigens associated with tumor cells but not on the animal's normal tissue—have been demonstrated in several animal species (*1*). These antigens were so named because they were detected by transplantation techniques. In order to detect antigens associated with tumor cells in man, one is restricted to immunological methods other than direct transplantation techniques. Consequently, evidence of tumor-associated antigens is more limited in man.

Using the indirect fluorescent antibody assay, Klein *et al.* (*2*) showed that on Burkitt lymphoma cells there was an "antigen" associated with the lymphoma cells and that this antigen was not detectable on normal bone marrow cells of the tested patient. Malmgren and Morton (*3*) observed antibodies in the serums of melanoma patients which gave relatively specific fluorescence with melanoma cells. Gold *et al.* (*4*), using immunoelectrophoresis, reported that carcinoma cells from the gastrointestinal tract possess antigenic determinants that are absent from normal cells of adult origin but are present in embryonic tissues and tumors from the gastrointestinal tract. Hellstrom *et al.* (*5*), using the colony inhibition assay, demonstrated tumor-associated antigens on neuroblastomas and some gastrointestinal tumors.

We now report results of mixed leukocyte culture tests with human leukemic leukocytes in vitro. Our data indicate that leukemic cells carry, and manifest in mixed leukocyte culture tests, antigens which normal lymphocytes do not manifest. These antigens could be leukemia-associated antigens.

In the mixed leukocyte culture test, peripheral blood leukocytes of one individual (A) are mixed with leukocytes (treated with mitomycin-C and designated subscript "m") of another individual (B). The untreated responding cells (A) enlarge and divide in response to foreign histocompatibility antigens present on the treated stimulating cells (B_m). After the cells are cultured for 7 days, incorporation of tritiated thymidine into acid-precipitable material is studied in allogeneic (AB_m) and isogeneic (AA_m) cell mixtures. The enhanced incorporation of tritiated thymidine in allogeneic mixtures (stimulation in mixed leukocyte culture test) is a measure of the response of A cells to foreign histocompatibility antigens of B_m cells and reflects the antigenic disparity of the two individuals at HL-A—the major histocompatibility locus in man (*6*). If the two test cells are identical at HL-A, then the cell mixtures AB_m and BA_m will not incorporate thymidine at a rate significantly higher than their respective controls, AA_m and BB_m (nonstimulation, identity in the mixed leukocyte culture test). If a mixture AB_m shows no stimulation, this result will only be accepted as indicating identity if, in suitable controls in the same experiment, the test cells (A) react as responding cells and the treated cells (B_m) act as stimulating cells.

In the human population, HL-A is a highly polymorphic locus, and thus individuals are generally heterozygous at this locus; two unrelated individuals will usually be heterozygous for different alleles. In a typical family, we can thus arbitrarily assign alleles *a* and *b* to the father and *c* and *d* to the mother. Segregation of these alleles yields four different combinations (*ac*, *ad*, *bc*, and *bd*) in the siblings, and 25 percent of ibling pairs should be identical at HL-A. Two such identical siblings will be heterozygous for the same alleles and will thus be identical at HL-A. Any siblings with dissimilar alleles will be reciprocally HL-A disparate. No sibling pairs should thus stimulate in one direction but not in the other when both parents are unlike heterozygotes. Consonant with these considerations, 28.2 percent of sibling pairs studied failed to stimulate in mixed leukocyte culture tests (*6*), and all of the nonstimulatory cell mixtures were nonstimulatory in both directions. No nonreciprocal stimulating pairs were found among siblings tested.

Using the mixed leukocyte culture test to choose donors for bone marrow transplantation we have studied families of 36 leukemic patients. Of these families, 30 were studied when the patient was in remission, that is, no leukemic cells appeared in a smear of the peripheral blood. In these families we found sibling pairs identical in the mixed leukocyte culture test in a frequency not significantly different from that found in normal sibling pairs (*6*). In all of the sibling pairs in these families that were identical in the mixed leukocyte culture test, there was no exception to the rule that the siblings were nonstimulatory in both directions. Six families with a leukemic member were studied at a time when the patient was in relapse, that is, between 30 and 100 percent of the leukocytes in a smear of peripheral blood