Influence of photoperiod on testicular recrudescence in high-temperature-maintained winter frogs

Treatments	Gonosomatic index		Relative number of I SPC cysts/ seminiferous tubule	
	January	February	January	February
Initial control	0.21±0.02	0.21±0.02	0.83±0.16	0.92±0.09
Dark	0.24±0.02	0.26±0.02[c]	0.98±0.10	1.08±0.13
LD 12:12	0.36±0.03[a,d]	0.35±0.02[a,d]	3.84±0.46[a,d]	3.56±0.59[a,d]
2-h light pulse				
09.00–11.00 h	0.19±0.02	0.33±0.03[a,d]	0.76±0.08	2.93±0.37[a,d]
11.00–13.00 h	0.25±0.04	0.30±0.02[a]	0.82±0.09	2.99±0.42[a,d]
13.00–15.00 h	0.25±0.04	0.28±0.02[a]	0.91±0.10	2.85±0.26[a,d]
15.00–17.00 h	0.23±0.04	0.27±0.02[b]	0.86±0.03	2.87±0.63[a,d]

[a] Significance of difference vs initial control ($p<0.01$); [b] significance of difference vs initial control ($p<0.02$); [c] significance of difference vs initial control ($p<0.05$); [d] significance of difference vs dark-treated frogs ($p<0.01$).

a significant increase in testis weight ($p<0.01$). In February the initial controls did not show any change in the testis as compared with January controls. The 2-h light pulse, as well as the LD 12:12 cycle however, induced a highly significant testicular growth. In comparison, although it was significantly higher than in the initial controls ($p<0.05$) the testis weight in dark-treated frogs was significantly lower than in the LD 12:12 and LD 2:22 (09.00–11.00 pulse) frogs ($p<0.01$).

In February, furthermore, except for frogs having a daily 2-h light pulse beginning at 09.00 h, the weight of LD 12:12 frogs' testes was far higher than in those given the short light pulse for 30 days beginning respectively at 11.00, 13.00 and 15.00 h.

The testis in January and February frogs usually contains numerous sperm bundles attached to Sertoli cells, some secondary spermatogonia cysts and a few spermatocytes. Histological examination of the testis in all experimental groups revealed evident signs of spermiation (presence of free sperm masses in the lumen of seminiferous tubules and efferent ductules).

Spermatogonial multiplication was enhanced in all groups. The differences were noted at the level of germinal cysts containing primary spermatocytes. Few spermatocyte cysts were found in dark-treated frogs or in those given the 2-h light pulse in January. In February the testis of all the 4 2-h light pulse groups contained numerous cysts containing primary spermatocytes. The number of such cysts was still greater in January and February frogs treated with 12:12 LD cycle (table).

These results indicate that in January the 2-h light pulse is not sufficient to induce testicular growth. On the contrary, in February, the 2-h light pulse, given at any time of the day, favoured the temperature-induced testicular growth, as in the LD12:12 frogs. In addition to this, the February dark-treated frogs did not show an increase in the number of primary spermatocytes.

It should be noted that in nature winter stasis terminates and testicular recrudescence sets in by the end of February or the beginning of March, when both day length and environmental temperature start increasing.

Although the importance of light in the temperature response of the testis has once again been shown, it is also evident that *R. esculenta* is not a strictly photoperiodic species. In fact, scanning the major part of the day at 2-h intervals showed that the photo-inducible phase, of the testicular rhythm during recrudescence (if it exists in *R. esculenta*), falls between 09.00 h and 17.00 h. But in this span of 8 h even a 2-h light pulse produced significant testicular growth with a probable peak at 09.00 h. A longer light pulse (12:12 LD), however, was still more stimulating which may mean that a longer light regimen might have an accumulative effect on testicular rhythm. In some teleosts it has been demonstrated that a circadian mechanism is involved for photoperiodic measurement[5,6]. Further experiments will have to be conducted to test whether such a mechanism also exists in the frog.

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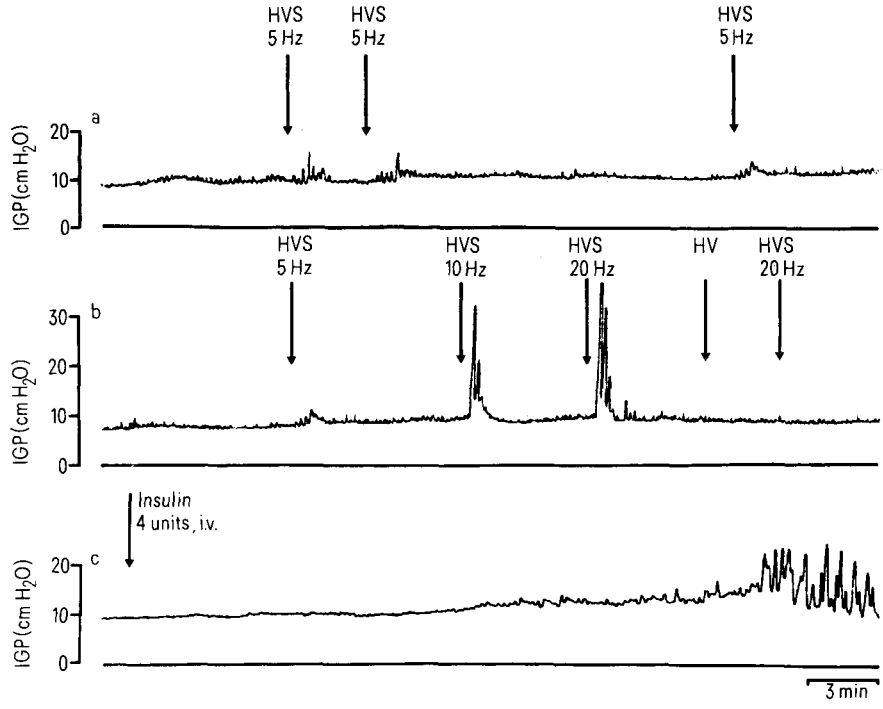
Reflex motility of the stomach evoked by electrical stimulation of the hepatic vagus nerve

T. Sakaguchi and Y. Miyaoka[1]

Department of Physiology, Niigata University School of Medicine, Niigata City (Japan), 21 April 1980

Summary. Electrical stimulation of the cranial end of the cut hepatic branch of the vagus nerve produced an increase in the motility of the empty stomach in the rat, whereas systemic injection of insulin produced a gradual increase in the motility.

It has been recognized that insulin and glucose alter gastric motility[2,3] and act by stimulating the neural glucose-sensitive mechanism located within the brain[4–6]. However, behavioural and neurophysiological studies have pointed out the existence of a neural mechanism responsive to glucose in the hepatic portal vessels[7–9]. Previously we noted that portal injection of glucose specifically affected the efferent activity of the vagus nerve innervating the stomach, and presumed that there might be a neural system modulating gastric function through hepatic vagal afferents and gastric



Changes in intragastric pressure induced by afferent stimulation of the hepatic vagus nerve (a, b) and venous administration of insulin (c). Arrows indicate the time of stimulation or administration. IGP, intragastric pressure; HVS, hepatic vagal stimulation; HV, hepatic vagotomy immediately above the stimulating electrode.

vagal efferents[10,11]. Recently we observed motility of the empty stomach in the rat that was enhanced by electrical stimulation of the hepatic branch of the vagus nerve.

Material and methods. Male Wistar rats weighing from 250 to 300 g were used. They were deprived of food for 22 h but allowed free access to tap water. The experiments were performed under pentobarbital sodium anaesthesia (45 mg/kg, i.p.). Rectal temperature was maintained about 34.5 ± 0.5 °C by a heating lamp. The motility of the stomach was evaluated by the changes in intragastric pressure, using a balloon method described earlier[3,4]. After a tracheal intubation, a balloon, introduced by way of the oesophagus into the gastric lumen, was inflated with warm water (34 °C) at an initial pressure of about 8 cm of water, and connected to a strain-gauge pressure transducer combined with an ink writing recorder. The elastic electrode for nerve stimulation, which could protect nerves from mechanical damage[12], was set on the cranial end of the cut hepatic branch of the vagus nerve, while the sympathetic nerve remained intact. The stimulation parameters were 6 V, 1 msec width (square wave for 15 sec) and the frequencies 5, 10 and 20 Hz.

Results and discussion. Stimulation of the hepatic vagus nerve (5 Hz) provoked a reliable increase in the motility of the stomach which was reproducible by repetitive stimulation of the nerve (figure a). The intensity of the response was electrically frequency-dependent in the range of 5–20 Hz, as shown in the figure b. Section of the hepatic branch of the vagus nerve at a level immediately above the exciting electrode abolished such responses to electrical stimulation, indicating that these responses could be ascribed to a change in central nervous activity (figure b). Insulin i.v. administered (4 µm) elicited a gradual increase in the motility (figure c). These findings were observed in 46 of 48 animals.

It has been assumed that hypoglycaemia increases the motility of the empty stomach by stimulating the neural glucose-sensitive mechanism in the CNS[4–6]. However, a mechanism sensitive to glucose has been revealed in the hepatic portal areas[7–9]. We previously found that efferent activity of the gastric vagus nerve was reduced by glucose infusion in the portal vein, whereas the resulting decrease in blood levels of glucose following an administration of insulin was accompanied by an increase in the activity[10]. These results, at least, indicated that there is a vagal pathway between the liver and the stomach. Concerning the possible physiological role of the pathway found, we recently suggested that there might be a system vagally modulating the secretion of gastric acid[11]. In the experiments presented in this paper, electrical stimulation applied to the cranial end of the cut hepatic vagus nerve caused an increase in the motility of the stomach. As a feature of afferent discharge arising from the hepatic vagus nerve, the inverse relationship between the level of glucose in the portal perfusion fluid and the frequency of the nerve discharge has been noticed[8]. We have in consequence interpreted the increasing frequencies of the electrical pulses given to the hepatic vagus nerve as a simulation of decreasing levels of glucose in the hepatic portal blood, a viewpoint which allows us to suggest that afferent information mediated by the hepatic vagus nerve may modulate the motility of the empty stomach.

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