Neural control and neurosensory functions of the liver

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In addition to the important roles of the mammalian liver in the control of homeostasis, such as the clearance, detoxification, inactivation and storage of fuels, it is subjected to various neural stimuli and appears to act as a sensory organ. The liver is in the ideal position to be involved in the control of metabolism and many changes are signalled to the central nervous system (CNS) by both neural and humoral pathways. Despite a considerable amount of research it is still difficult to draw firm conclusions from the available evidence in view of the often controversial reports in the literature. The neural mechanisms, especially the sensory functions of the liver, have proved to be particularly difficult to interpret for the following reasons: (1) lack of clear knowledge of the location and routes of hepatic nerves in different species; (2) difficulty in employing conventional histological and electrophysiological techniques; (3) different experimental approaches resulting in discrepant results; (4) uncertainty of relating effects of neural activation directly to the liver and the possibility that other splanchnic organs are involved, especially the adrenal medulla and pancreas.

Combinations of physiological, histological and behavioural approaches need to be made to advance our understanding of the integration of the liver into the whole complex of neural and humoral systems involved in the control of metabolism.

Neuroanatomy of the liver

Before studies of the roles of the nervous system in liver function can be considered, it is necessary to understand the nerve supply to the liver. The mammalian liver is mainly innervated by the branches of the vagus nerves (parasympathetic) and the splanchnic nerve(s) (sympathetic) of the autonomic nervous system (Fig. 1). As branches of these nerves enter the liver at the porta hepatis, they form a distinct hepatic plexus with ramifications around the hepatic portal vein and, particularly, the hepatic artery (Lautt, 1983). There are considerable differences between species in the origins and distribution of hepatic nerves, as reviewed by Metz & Forssman (1980).

The hepatic nerves carry both afferent and efferent fibres. Postganglionic, sympathetic efferents originate in the coeliac ganglion, whereas parasympathetic, postganglionic fibres come from ganglia situated in the liver. Distribution of nerves within the liver is variable and conflicting reports make interpretation difficult, even though the first studies were reported as early as 1869 by Pflüger (Forssman, 1980). It is generally agreed, however, that most fibres are associated with the hepatic blood vessels and terminate on smooth muscle fibres, suggesting a

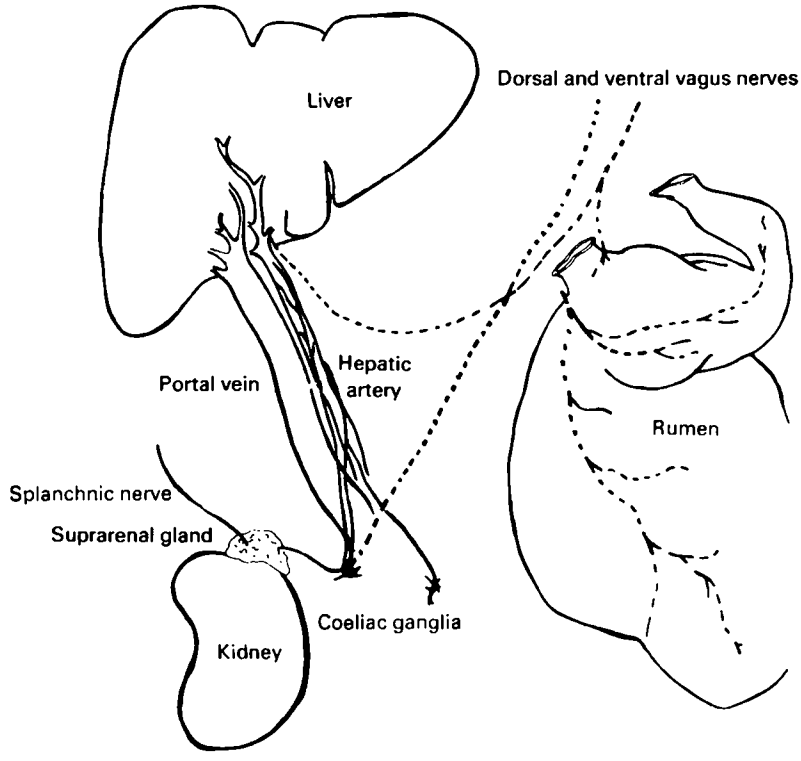

Fig. 1. Autonomic innervation of the stomach and liver in sheep.

vasomotor function (Nicolescu, 1958). Other terminations are near the spaces of Disse and on the surface of hepatocytes (Forssman & Ito, 1977).

Electron-microscope studies have confirmed that most nerves in the liver are associated with vessels, with considerable variation between species. For example, in the rat and mouse the innervation is reported to be restricted to the triads (of blood and bile vessels) with no apparent parenchymal innervation, whereas the guinea-pig, rabbit, cat and dog show well-developed parenchymal, intralobular innervation. Despite the evidence from light microscopy of fibres terminating on hepatocytes (Skaaring & Bierring, 1977), electron-microscope work has provided conflicting reports on intralobular innervation, mainly stemming from the difficulty of differentiating neural tissue from connective tissue fibres which take up the stains normally used to show nerves. We have been examining sections of ovine liver under transmission electron microscopy and so far have been unable to see nerve endings in the spaces of Disse which resemble the dense core vesicles reported by Forssman & Ito (1977) in the rabbit, cat and tree shrew.

A further difficulty in the study of liver innervation is the lack of reliable methods for differentiating adrenergic from cholinergic fibres under electron microscopy (McCuskey, 1980).

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Nervous control of liver function

Central nervous control. There is less known about the central sites of control than there is about the effects of stimulating or sectioning peripheral nerves although peripheral effects are presumed to be the result of signals initiated in the brain.

The ventromedial nucleus of the hypothalamus (VMH) plays a very important role in the control of metabolism, so it is not surprising that stimulation of this area of the brain can affect liver metabolism. Using electrical stimulation of the VMH, Shimazu *et al.* (1978) showed a rapid increase in hepatic phosphorylase (*EC* 2.4.1.1) activity, which also occurred with chemical stimulation of the VMH with noradrenaline (Matsushita & Shimazu, 1980). This latter effect was blocked if the VMH site was previously injected with propranolol (a β-adrenergic antagonist) but not with phentolamine (an α-adrenergic blocker). Stimulation of the lateral hypothalamic (LH) area, either electrically or with cholinergic agonists, stimulated glycogen synthase (*EC* 2.4.1.11) activity in the liver (Matsushita *et al.* 1979), the cholinergic effect being blocked by pre-injection with atropine (a cholinergic blocker). This suggests that the dual-centre–dual-neurotransmitter theories of the control of voluntary food intake might also be applicable to the central nervous control of liver function. It might indeed be that the VMH and LH primarily control endocrine and metabolic functions, with secondary effects on food intake.

Shimazu (1962, 1964) stimulated the VMH and LH electrically and noted increases in liver tryptophan 2,3-dioxygenase (*EC* 1.13.11.11) activity; in the former case the saturation of the enzyme with the cofactor was much greater than with LH stimulation. Cholinergic application to the LH also increased the liver activity of tyrosine aminotransferase (*EC* 2.6.1.5), an effect which could be prevented by pre-injection with atropine or by bilateral vagotomy (Shimazu, 1981).

Peripheral nervous control. It is known that blood flow to and through the liver is under nervous control (see Lautt, 1983) and that this might have indirect effects on metabolism. However, this section will concentrate on direct effects of efferent nerves on metabolism in the liver and those effects mediated by pancreatic and adrenal medullary hormones.

Electrical stimulation of the splanchnic nerve causes release of glucose by the liver, both indirectly by releasing adrenaline from the adrenal medulla and by direct effects on hepatocytes. Stimulation of nerves in the hepatic plexus, to avoid involvement of adrenal glands and pancreas, results in an immediate, short-lived glycogenolysis. Although such local stimulation probably involves both sympathetic and parasympathetic fibres, it is most probably the former which are involved in the glycogenolytic response, via α-adrenergic receptors. Simultaneous stimulation of the vagus nerves blocks the effects of splanchnic-nerve stimulation (Shimazu, 1981).

Noradrenaline infusion into rabbits stimulated a rise in the blood concentration of glucose in a manner similar to splanchnic-nerve stimulation and both effects were blocked by (pre)treatment with an α-adrenergic blocker (Proost *et al.* 1979).

There is, however, conflicting evidence from rabbits that neither α- nor β-blockers prevent the phosphorylase activation response to nerve stimulation of the liver and conversely that the response to noradrenaline is blocked by α-blockers (Shimazu & Usami, 1982); unfortunately these authors did not measure blood glucose concentrations. Edwards (1972) showed that the effect of splanchnic stimulation on hepatic glycogenolysis was not modified by adrenalectomy and pancreatectomy, thus supporting direct nervous affects on the liver as being important in addition to indirect effects via adrenalin and pancreatic hormones.

Vagal stimulation causes increased glycogen synthesis in the liver, the rate of uptake of radioactive glucose being increased during stimulation of the vagus but reduced by splanchnic stimulation (Shimazu & Fujimoto, 1971). Conversely, vagotomy reduces the rate of glycogen synthesis after administration of a load of glucose (Mondon & Burton, 1971).

Nerve inactivation is an alternative to nerve stimulation as a technique for studying the role of the nervous system in the control of liver function and the most direct way of inactivating a nerve pathway is to cut the nerves involved. Sectioning of the left splanchnic nerve affects both liver and pancreas and it is not easy to interpret results of experiments which used this technique (Jarhult *et al.* 1980). Nerve sectioning close to the liver did not prevent the hyperglycaemia caused by haemorrhage in cats, nor did selective hepatic sympathectomy performed by hepatic portal vein infusion of 6-hydroxydopamine (Lautt *et al.* 1982). However, when coupled with adrenalectomy, these procedures did prevent the hyperglycaemia (Lautt, 1983), showing that the stimulus of haemorrhage exerted its effects on the liver via adrenal activation.

The effects of sympathetic stimulation involve an increase in the activity of phosphorylase both in the rat (Preiksaitis & Kunos, 1979) and in the sheep (Anil *et al.* 1987).

The response of hepatic enzyme activity to sympathetic stimulation is variable, being reduced by high levels of circulating insulin and increased by glucagon (Beckh *et al.* 1982). Further evidence for pancreatic involvement in responses to general stimulation of the splanchnic nerve comes from the observation that stimulation at a point before the bifurcation to the pancreas and liver leads to decreased insulin and increased glucagon secretion, as well as the anticipated hyperglycaemia, while stimulation close to the liver (to exclude the pancreas) gives no hormonal changes and a greatly reduced stimulation of glycogenolysis (Jarhult *et al.* 1980). However, Lautt (1980) reported much greater elevation of blood glucose concentration following stimulation, similar to the close hepatic stimulation of Jarhult *et al.* (1980), again without hormonal response.

Exercise induces glycogenolysis which in rats occurs before any measurable change is detectable either in glucagon secretion or in liver concentration of cyclic AMP (Winder *et al.* 1979), leaving direct effects of the nervous system on the liver as the most likely route. In glycogen-depleted (fasted) rats, both glucagon and adrenaline concentrations in plasma rose more quickly after the onset of exercise

than in free-fed rats, therefore both nervous and hormonal mechanisms are involved in this case.

Insulin-induced hypoglycaemia causes glycogenolysis, possibly involving catecholamine secretion (Sacca *et al.* 1977), while cellular glucoprivation induced with 2-deoxyglucose in man leads to hyperglycaemia without changes in plasma concentrations of glucagon or catecholamines, suggesting that direct effects of the nervous system on the liver are involved. Prolonged insulin treatment reduces the number of insulin receptors in liver. Denervation of the liver prevented this effect while the reduction in adipose-tissue receptor numbers and activity were not affected (Komissarenko *et al.* 1982).

Cholesterol synthesis in the liver is depressed by ligation of the coeliac nerve (principally sympathetic) while sectioning of both vagus nerves (parasympathetic) stimulates cholesterol synthesis (Shanygina *et al.* 1981). The lipoprotein content of liver is also affected by these two manipulations, in the same direction as cholesterol. There has been a little work on the role of the nervous system in the control of amino acid metabolism in the liver. Black & Reis (1971) showed that hepatic sympathetic nerves are necessary for the rhythmic changes in hepatic tyrosine aminotransferase activity. Vagotomy reduces the early increase in ornithine decarboxylase (*EC* 4.1.1.17) activity that normally follows surgical removal of part of the liver (Lamar & Holloway, 1977). This might not be a direct effect in view of the fact that the vagus innervates the pancreas as well as the liver.

The general conclusion can be drawn that the sympathetic and parasympathetic efferent divisions to the liver exert opposite effects on its metabolic activity, the former causing glycogenolysis.

Sensory functions of the liver

In a histological study of the liver, Seto (1963) declared it to be without sensation while Tsai (1958) described three types of presumptive sensory nerve endings in human and dog liver. Most of the abdominal vagal fibres are afferent and unmyelinated; the hepatic nerves are mostly unmyelinated and may therefore be mainly afferent.

Non-metabolic receptors. Haberich *et al.* (1965) suggested that peripheral osmoreceptors situated in the portal vein or in the liver were complementary to the central osmoreceptors.

Andrews & Orbach (1975), recording from the hepatic nerves of the rabbit, found that solutions of varying osmolarity altered the rates of discharge of afferent fibres. Information from the hepatic receptors may be carried, at least in part, by the hepatic branch of the vagus nerve, as Niijima (1969) found a direct relation between the osmolarity of the liver perfusate and the frequency of afferent vagal discharges from the isolated guinea-pig liver. Some studies, however, failed to demonstrate the existence of hepatic osmoreceptors (for review, see Lautt, 1980).

Hepatic ion receptors, rather than osmoreceptors, have also been implicated because portal infusions of hypertonic saline produced greater increases in sodium excretion compared with infusions into the general circulation in the dog (Daly

et al. 1967). Furthermore, electrophysiological recordings showed changes in the activity of the afferents with saline but not with equiosmotic solutions of glucose or mannitol (Andrews & Orbach, 1974). In another study, the single unit activities of neurones in the hypothalamic area were altered in response to intraportal administration of hypertonic, but not isotonic, saline (Schmitt, 1973). Aisman & Finkinstein (1976) observed increases in potassium excretion, which could be abolished by vagotomy, following infusion of potassium chloride solutions into the hepatic portal vein.

There is also experimental evidence for baroreceptors in the portal vein and in the liver: Ohm & Haberich (1969) reported changes in urine flow-rate inversely related to changes in portal pressure. Although these responses were not altered by vagotomy, they were abolished by renal nerve blockade. Hepatic congestion also has effects on the kidney such as reduced urine flow and Na excretion (see Lautt, 1980).

Metabolic receptors. Receptors sensitive to metabolite availability have been thought to exist in the brain for many years and Mayer's (1955) glucostatic theory for the control of voluntary food intake suggested that glucoreceptors are located in the hypothalamus. However, in recent years there has been more emphasis on the role of the liver as a site for metabolic receptors. Russek (1963) was the first to suggest a sensory role for the liver in the control of food intake and that free glucose in the liver acted as a satiety signal. It remains unclear whether the postulated receptors are sensitive only to glucose or to other metabolites as well. Intraportal infusions of glucose have suppressed food intake in dogs (Russek, 1970), rats (Booth & Jarman, 1976), rabbits (VanderWeele *et al.* 1976) and chickens (Shurlock & Forbes, 1981). In contrast, some studies have failed to demonstrate an effect on feeding of glucose infusion into the liver. Differences in experimental design (e.g. whether or not pre-fasting was used) and surgical technique (e.g. whether the tip of the catheter was in the portal vein or a mesenteric vein) as well as species may account for the discrepancies. This area is fully discussed by Sawchenko & Friedman (1979) and Novin (1983).

Although glucose does not affect food intake in ruminants, even when infused into the portal vein, propionate (the major precursor of glucose in ruminant liver) causes a marked depression in intake, especially when given into the portal vein; total section of the hepatic plexus of nerves abolishes this effect (Anil & Forbes, 1980).

Electrophysiological evidence for hepatic sensitivity to glucose has come from the work of Niijima (1969) who, using the isolated perfused guinea-pig liver preparation, recorded the electrical activity of the hepatic branch of the vagus nerve. He found that additions of glucose to the perfusing medium depressed the firing rate of the recorded units, although other hexoses had no effect. Consistent with this was the finding of Schmitt (1973) who reported that there were units in the hypothalamus of rats which responded to portal infusions of glucose solutions. The response was greater in the evening, when spontaneous activity in this region is at its peak, than at other times of day.

Niijima (1983) observed that the discharge rates of hepatic afferent fibres increased with insulin treatment and decreased in response to cholecystokinin or glucagon.

Although it is widely believed that the hepatic vagus carries signals from the hepatic receptors, information might also be carried in sympathetic nerves. In Schmitt's (1973) study, the response of the lateral hypothalamic units to intraportal glucose was abolished by splanchnotomy. Similarly, in our experiments with sheep, splanchnic-nerve sectioning was sufficient to prevent the effect on feeding of propionate infused into the portal vein (Anil & Forbes, 1984). In an attempt to temporarily block neural transmission in the splanchnic nerves we have applied local anaesthetic to these nerves via chronically implanted cuffs and found reversal of the effect of intraportal propionate on feeding (Fig. 2); selective vagotomy close to the liver also abolishes the propionate-induced depression in feeding in sheep (M. H. Anil and J. M. Forbes, unpublished results, Fig. 2) so that, once again, more than one route from the liver to the CNS is implied.

Other research indicates that the liver detects changes related not only to glucose metabolism but also to the supply of oxidizable fuels in general (see Sawchenko & Freidman, 1979). In a recent study, Langhans *et al.* (1985) reported that the hypophagic effects of a variety of metabolites such as glycerol, 3-hydroxybutyrate, malate, lactate or pyruvate originate in the liver as their effectiveness is dependent on intact vagal branches to the liver. Lysine infused into the portal vein depresses feeding in chickens and this can also be prevented by vagotomy local to the liver and pancreas (Rusby, 1985).

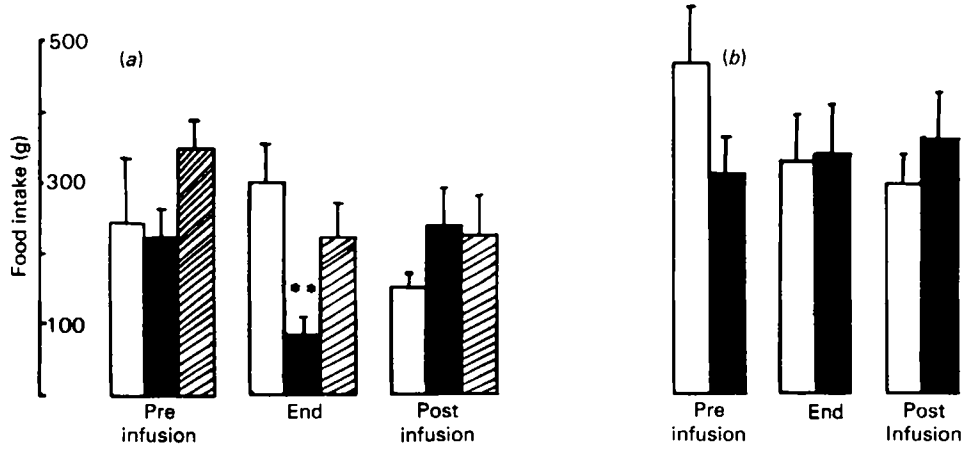

Fig. 2. Effects of intraportal infusion on food intake of (*a*) intact and (*b*) vagotomized sheep. (□), Saline; (■), sodium propionate at 1·2 mmol/min for 3 h; (▩), splanchnic nerve blockaded with anaesthetic and propionate infused into the portal vein. Preinfusion, intake (g) during the 90 min before infusion; End, intake during the 180 min infusion; Postinfusion, intake during 150 min after the infusion. Values are means, with their standard errors represented by vertical bars, for five sheep per group. $^{**}P<0.01$.

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The neural pathways from the viscera to the CNS, which are involved in the control of ingestion, have been discussed in detail by Norgren (1983). Histochemical studies have shown that the hepatic vagal afferents project to the nucleus of the tractus solitarius in the brain stem. The evidence suggests that the gustatory and visceral axons terminate in the nucleus of the tractus solitarius and this is a potential site for interactions between information from the special senses and the viscera.

Other receptors. Other receptors studied in the liver include those for temperature and pain. Heating the liver reduced food intake and splanchnic nerve sectioning abolished this effect (Sawchenko & Friedman, 1979). In liver disease, the pain felt by most patients cannot be due to the stretching of the ligaments as it is still present in the absence of swelling (Lewis, 1951). As well as the phrenic nerve, the splanchnic nerve may also be involved in the sensation of pain (see Sawchenko & Friedman, 1979).

Another area of interest is the possible role of the vagus in liver regeneration. The massive hyperplasia which follows hypertrophy after partial hepatectomy, seems to be dependent on an intact vagal supply; without the latter the rise in DNA activity is slowed (Lamar & Holloway, 1977).

There remains the possibility that some of the effects of hepatic denervation mentioned in this section might be a result of cutting efferent as well as afferent fibres.

Conclusions

Evidence continues to accumulate of very significant nervous pathways between the CNS and liver which augment the well-established humoral pathways. As a major function of the liver is to attenuate the fluctuations of nutrients coming from the digestive tract it is well placed to inform the CNS of impending changes in nutrient supply, and to be one target of neural pathways which are involved in the responses to variations in nutrient supply to the rest of the body. Further progress will depend on close attention to the methodological problems which have made it difficult to interpret much of the available evidence in this field.

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