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UPTAKE OF 5-HYDROXYTRYPTAMINE AND ADRENALINE IN THE LIVER

BY

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A dose of 5-hydroxytryptamine caused a greater contraction of the cat nictitating membrane when injected into the jugular vein than when injected into the portal vein. The difference is attributed to uptake or destruction of 5-hydroxytryptamine by the liver. The effects of portal and jugular injections became equal after administration of the monoamine oxidase inhibitors iproniazid or harmaline. Isoniazid, which does not inhibit monoamine oxidase, did not have this effect. Similarly, the effect of a portal injection of adrenaline was less than the effect of an equal jugular injection of adrenaline. Iproniazid and harmaline increased the effect of a portal injection, but not to equal the effect of a jugular injection. Isoniazid had no effect. Pyrogallol did not alter the ratio between the effects of portal and jugular injections of adrenaline, but increased the responses to both.

The portal venous blood of dogs was shown by Toh (1954) to contain about three times as much 5-hydroxytryptamine as did arterial blood. Erspamer & Testini (1959) found that hepatic vein blood contained more 5-hydroxytryptamine than did blood from the inferior vena cava, but the difference was considerably less than Toh had found between portal and arterial blood. In addition, other observations suggest that 5-hydroxytryptamine may be taken up or destroyed while passing through the liver. Blaschko (1952) reported that liver monoamine oxidase broke down 5-hydroxytryptamine; Sjoerdsma, Smith, Stevenson & Udenfriend (1955) showed that metabolism of 5-hydroxytryptamine by the mitochondrial portion of liver cells was inhibited by iproniazid and that iproniazid enhanced the effects of 5-hydroxytryptamine injected intraperitoneally. The observation of Ewins & Laidlaw (1913) that tryptamine was broken down to indolyl-3-acetic acid by the rabbit perfused liver also suggested that 5-hydroxytryptamine might be handled in the same way.

Catechol amines also may be inactivated by the liver. Dawes (1946) and West (1948) injected equal doses of adrenaline and noradrenaline into the jugular and portal veins and found that portal injections caused smaller contractions of the cat nictitating membrane than did jugular injections. Similar experiments comparing the effects of portal and jugular injections of 5-hydroxytryptamine are reported here.

METHODS

Cats of either sex were anaesthetized with sodium pentobarbitone (45 mg/kg intraperitoneally). The effects on the nictitating membrane of portal and jugular injections of

5-hydroxytryptamine were compared. Injections into the portal vein were made through a polyethylene cannula inserted into the splenic vein. Injections into the jugular vein were made either directly with a fine hypodermic needle or through a polyethylene cannula tied into the vein. Drugs were washed in with 1 ml. of 0.9% saline when injections were made through a polyethylene cannula. Contractions of the nictitating membrane were recorded either isotonically, with gimbal levers at a tension of 5 g and 25-times amplification, or isometrically with a Grass force-displacement transducer with an initial tension of 1 g. 5-Hydroxytryptamine creatine sulphate and (−)-adrenaline bitartrate were used; quantities refer to the free bases. Iproniazid phosphate (Marsilid, 100 mg/kg), isoniazid (Rimifon, 100 mg/kg), harmaline (20 to 40 mg/kg) and pyrogallol (100 mg/kg) were injected into the jugular vein in some experiments.

RESULTS

In twelve experiments the contraction of the nictitating membrane due to 5-hydroxytryptamine was greater when the dose was given into the jugular than when it was given into the portal vein (Fig. 1a). The ratio between portal and

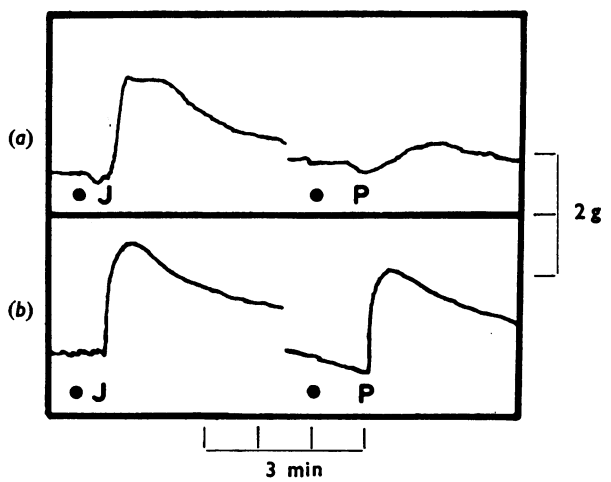

Fig. 1. Isometric recording of contractions of cat's nictitating membrane after 5-hydroxytryptamine (35 μg/kg) injected into the jugular vein (J) and into the portal vein (P). In (*a*) before, and in (*b*) 120 min after iproniazid (100 mg/kg).

jugular doses causing equal contractions was usually about 2:1. The difference was more apparent with small or moderate doses (10 to 50 μg/kg).

There was a delay of 60 to 90 sec after portal injection before contraction occurred, but only 30 sec after jugular injection. The longer delay after portal injection suggested that the smaller response might be due to slowing of the release of the drug into the general circulation after distribution in the large vascular bed of the liver. However, when the jugular injection was made slowly, over a 2 min period, its effect was not reduced to equal the effect of portal injection. It therefore appeared probable that a part of the 5-hydroxytryptamine injected by the portal route was taken up or destroyed by the liver.

In view of the work of Sjoerdsma *et al.* (1955) showing that the effects of intraperitoneal injections of 5-hydroxytryptamine were increased by iproniazid, it was thought that monoamine oxidase inhibitors might prevent the loss of 5-hydroxytryptamine in the liver. If this should occur, a role of dilution or delay in a large vascular bed could be excluded. A monoamine oxidase inhibitor was injected into the jugular vein in seven experiments. In four experiments with iproniazid (100 mg/kg) the effects of portal and jugular injections of 5-hydroxytryptamine became equal after 90 to 150 min had elapsed (Fig. 1). Responses to jugular injection were unchanged. The passage of 5-hydroxytryptamine through the liver did not appear to be accelerated, for the delay between portal injection and contraction was not shortened. Harmaline, a more rapidly acting monoamine oxidase inhibitor with a shorter duration of action (Pletscher, Besendorf, Bächtold & Gey, 1959), was used in three experiments. The responses to portal and jugular injections became equal within 5 min of injecting harmaline (20 to 40 mg/kg) and in two of the three experiments reverted to their original relation within 90 min (Fig. 2). In contrast, responses

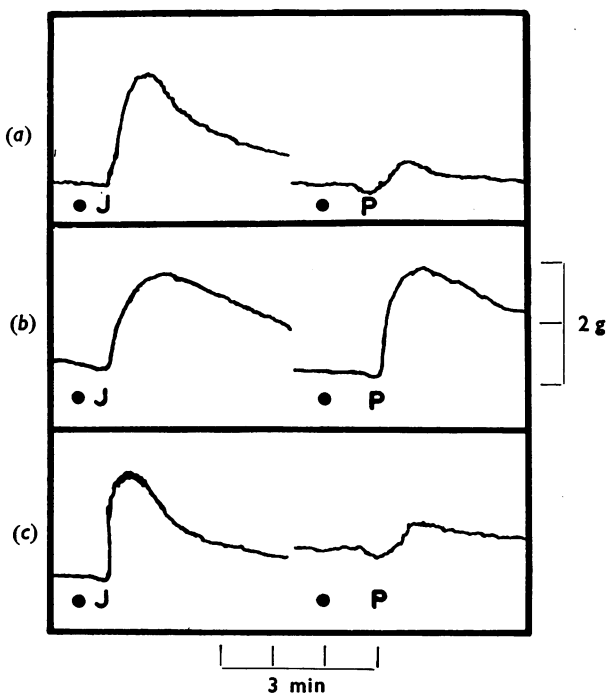

Fig. 2. Isometric recording of contractions of cat's nictitating membrane after 5-hydroxytryptamine (35 μg/kg) injected into the jugular vein (J) and into the portal vein (P). In (*a*) before, in (*b*) 20 min after, and in (*c*) 90 min after harmaline (40 mg/kg).

to portal and jugular injections were unchanged in two experiments with isoniazid (100 mg/kg), a congener of iproniazid without inhibitory action on monoamine oxidase.

It has been shown many times that adrenaline also has a greater systemic effect when injected into a jugular or saphenous vein than when injected into the portal vein (see Dawes, 1946). Experiments were therefore done to find whether iproniazid and harmaline affected portal injections of adrenaline as well as 5-hydroxytryptamine. Iproniazid (100 mg/kg) was injected into the jugular vein in three experiments, harmaline (20 mg/kg) in two experiments and isoniazid (100 mg/kg) in two experiments. In the experiments with iproniazid and harmaline the effects of portal injections of adrenaline (3 to 6 μg/kg) were increased, but never to more than half the magnitude of those of an equal dose of adrenaline given by the jugular vein. The increase in the effect of portal doses of adrenaline developed at the same time after injection of iproniazid and harmaline as did equalization of responses to 5-hydroxytryptamine. The effects of jugular injections of adrenaline were not increased. The addition of an *O*-methyl-transferase inhibitor, pyrogallol (100 mg/kg), enhanced the effects of both jugular and portal injections of adrenaline but did not alter the ratio which had been established by the monoamine oxidase inhibitors. Isoniazid (100 mg/kg) did not increase the effect of portal injections of adrenaline.

DISCUSSION

The results indicate that 5-hydroxytryptamine is taken up or inactivated by the liver. It is unlikely that the loss of effect of a portal dose of 5-hydroxytryptamine is due to distribution through a large volume of blood, thereby delaying its passage to the arterial blood and allowing more opportunity for its destruction. Two facts point to this conclusion. First, the effect of a jugular injection was not correspondingly reduced when the injection was made slowly over a 2 min period. Second, the monoamine oxidase inhibitors iproniazid and harmaline each increased the effect of portal injections without altering the delay between injection and response.

The rates of onset of the actions of iproniazid and harmaline in equalizing the effects of portal and jugular injections of 5-hydroxytryptamine are similar to the rate of onset of monoamine oxidase inhibition by these drugs in rats (Pletscher *et al.*, 1959). The monoamine oxidase inhibiting activity of harmaline in these rats fell to 50% of the maximal effect within 4 hr. The short duration of the effects of harmaline in the present experiments agrees with these observations. The experiments do not establish that iproniazid and harmaline increase the action of portal injections of 5-hydroxytryptamine by virtue of their inhibitory activity on monoamine oxidase, although the failure of isoniazid to have the same effect as iproniazid suggests that monoamine oxidase inhibition may be involved. The results could be explained equally well by iproniazid and harmaline preventing the uptake of 5-hydroxytryptamine by the liver cells, rather than inhibiting its destruction.

Dawes (1946) found that contraction of the cat nictitating membrane due to portal injection of adrenaline was increased by amidines or guanidines injected simultaneously into the portal vein. The contraction then approached but did not equal the contraction due to the same dose of adrenaline injected into the jugular vein. He suggested that the reduction of adrenaline uptake by the liver might be due

to the drugs interfering with penetration of adrenaline into the liver cells. Inhibition of monoamine oxidase was not believed to play a role. Although some of the drugs which enhanced the effect of portally administered adrenaline were monoamine oxidase inhibitors, other drugs had monoamine oxidase inhibiting activity but lacked any potentiating action. However, the absence of this effect might have been due to a slow onset of action of the monoamine oxidase inhibitor or to its failure to penetrate to the site of uptake or destruction of adrenaline. The present experiments with iproniazid, harmaline and isoniazid showed potentiation after treatment with the monoamine oxidase inhibitors only. Although only three drugs were used in the present study, the experiments indicate a correlation between inhibition of monoamine oxidase activity and reduction of the disappearance of portally injected adrenaline and 5-hydroxytryptamine. However, the rate of disappearance of 5-hydroxytryptamine and adrenaline in a single passage through the liver is much greater than would be expected from experiments *in vitro* on their rate of destruction by monoamine oxidase. Moreover, liver monoamine oxidase is contained almost exclusively in the mitochondria of the liver cells (Cotzias & Dole, 1951). It seems unlikely that, in the short time available, enough 5-hydroxytryptamine or adrenaline would enter the mitochondria to account for the disappearance of a high proportion of the amines. It is conceivable that monoamine oxidase inhibitors have also properties which permit them to combine with sites in the liver to which 5-hydroxytryptamine and adrenaline passing through the liver may normally become bound without necessarily being destroyed. Occupation of such binding sites by the monoamine oxidase inhibitors might explain the potentiating action of these drugs.

The failure of pyrogallol, given in a dose which potentiated the action of adrenaline, to alter the ratio between responses to portal and jugular injections of adrenaline suggests that liver *O*-methyl-transferase is not responsible for the rapid removal of adrenaline from the blood passing through the liver. These experiments suggest also that potentiation of the action of adrenaline by pyrogallol is not due to inhibition of *O*-methyl-transferase in the liver, although inhibition of the enzyme near the smooth muscle cells is not excluded.

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