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# Hypersensitivity of mesenteric veins to 5-hydroxytryptamine- and ketanserin-induced reduction of portal pressure in portal hypertensive rats

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**1** Isolated superior mesenteric veins from portal hypertensive rats were 3 to 10 times more sensitive to 5-hydroxytryptamine (5-HT) and 3 times less sensitive to (−)-noradrenaline than veins from sham-operated rats. The sensitivity to vasopressin did not differ in the 2 groups.

**2** Ketanserin competitively antagonized the effects of 5-HT in superior mesenteric veins and portal veins with high affinity ($K_B$ values 0.1–0.3 nM), as expected for 5-$HT_2$-receptors. The affinity of ketanserin for 5-$HT_2$-receptors was similar in veins from normal, sham-operated or portal-hypertensive rats.

**3** Intraportal injections of low doses of 5-HT caused increases in portal pressure which were more pronounced in portal hypertensive rats than in sham-operated rats and were blocked by 0.3 mg $kg^{-1}$ ketanserin in both groups. Ketanserin 0.3 mg $kg^{-1}$ did not block the portal pressor response to (−)-noradrenaline in either group of rats.

**4** In portal hypertensive rats but not in sham-operated rats, 0.3 mg $kg^{-1}$ ketanserin caused decreases in portal pressure, portal flow and cardiac output, as estimated by radioactive microspheres.

**5** The reduction in portal pressure caused by ketanserin was due mainly to a decrease in portal venous inflow secondary to a decreased cardiac output. The reduction in cardiac output, which was observed only in the portal hypertensive rats but not in sham-operated rats, is consistent with venous dilatation and pooling of blood in the portal venous system. The venous pooling could be secondary to the blockade of 5-$HT_2$-receptors in the portal venous system. It is proposed that ketanserin should be explored for the treatment of patients with portal hypertension.

## Introduction

Chronic portal hypertension can be induced in rats by partial ligation of the portal vein (Halvorsen & Myking, 1974). Within a short period of time the initial high and fixed resistance induced by the ligature around the portal vein is markedly reduced by the dilatation of pre-existing portal-systemic collaterals (Groszmann *et al.*, 1982; Sikuler *et al.*, 1985). In this model we have shown that splanchnic blood flow is markedly enhanced and that this increase in blood flow plays an important role in the maintenance of portal hypertension (Vorobioff *et al.*, 1983). However, when this increased splanchnic blood flow is reduced by different manoeuvres (haemorrhage, β-blockers, nitroglycerin, etc.) to normal values the portal hypertension persists. This persistence of the portal hypertensive state, despite a reduction in portal blood flow, is due to a marked increase in portal-collateral resistance (Kroeger & Groszmann, 1985a, b; Kravetz *et al.*, 1986). It has recently been suggested that, even during the high splanchnic blood flow state, the portal-collateral resistance is inappropriately high for that elevated blood flow (Kroeger & Groszmann, 1985a; Sikuler & Groszmann, 1986). Conceivably, the increased portal collateral resistance could be due to an enhanced constrictive response to noradrenaline and/or other naturally occurring venoconstrictive factors. 5-Hydroxytryptamine (5-HT) is a powerful venoconstrictor that has been shown to increase portal resistance in normal dogs (Richardson & Withrington, 1977). This study was designed to investigate whether noradrenaline and/or 5-HT could be involved in maintaining high portal-collateral resistance in portal hypertension. To this end we investigated the venous

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reactivity to noradrenaline and 5-hydroxytryptamine in isolated superior mesenteric veins of portal hypertensive and sham-operated rats.

We report that mesenteric veins of portal hypertensive rats were sensitized to 5-HT. To investigate whether or not 5-$HT_2$-receptors were involved we used the 5-$HT_2$-receptor antagonist ketanserin (Van Nueten *et al.*, 1981). We found that ketanserin lowered portal pressure and we therefore studied the haemodynamic effects of ketanserin in portal hypertensive and sham-operated rats. Preliminary accounts of this work were communicated by Groszmann & Kaumann (1983), Cummings *et al.*, (1985) and by Kaumann to the International Workshop on 'The Hemodynamic Aspects of Liver Disease', in Helsinki, on August 31st, 1985.

## Methods

Male Sprague-Dawley rats (Harlen Sprague-Dawley Laboratories, Indianapolis, IN) were housed in plastic cages and allowed free access to rat diet (Ralston-Purina, St. Louis, MO) and water until the time of study.

Portal hypertension was experimentally produced under ketamine anaesthesia (100 mg $kg^{-1}$ i.m.) with controlled portal vein constriction (Chojkier & Groszmann, 1981). Briefly, a single 3–0 silk ligature was tied around both the surgically isolated portal vein and an adjacent 20 gauge blunt-tipped needle. The needle was then removed and the portal vein allowed to re-expand. The abdominal operative incision was then closed using a double layer.

In the sham-operated rats, the portal vein was similarly isolated but no ligature was used. The abdomen was similarly closed. Rats were matched by weight and then randomly assigned to receive portal vein constriction or a sham operation. Rats were studied 2 weeks after the operation.

### *Isolated veins and arteries*

Rats were anaesthetized with ether. After opening the abdomen via a parasagital incision, the portal pressure was measured using a PE-50 catheter inserted into the superior mesenteric vein. The catheter was connected to a Statham transducer. The superior mesenteric vein and the portal vein were quickly removed with a 7/0 surgical silk thread attached to the distal end of each vessel. In some rats the mesenteric artery and the abdominal aorta were also removed. The tissues were dissected at room temperature in an oxygenated solution containing (mM): NaCl 137, KCl 2.7, $MgCl_2$ 0.5, $CaCl_2$ 1.4, $NaHCO_3$ 11.9, $NaH_2PO_4$ $2H_2O$ 0.4, $Na_2EDTA$ 0.04 and glucose 5; water was deionised and redistilled. The tissues were freed of fat and connective tissue. The tissues were set up in pairs in an apparatus with a 50 ml organ bath (Blinks, 1965) containing the above solution gassed with 95% $O_2$ and 5% $CO_2$. Experiments were carried out at 37°C. Each organ bath always contained a tissue from a portal hypertensive rat and the same type of tissue from a sham-operated rat. Each tissue was clamped at the proximal end and tied at the distal end to a stainless steel hook attached to a strain gauge force transducer (Swema SG4-45, Sweden) connected to a Grass 7D polygraph. The veins were mounted isometrically in the longitudinal direction with a preload of 5 mN. Helicoids of mesenteric artery and aorta were prepared and mounted isometrically with preloads of 5 mN and 25 mN, respectively.

A 128 mM KCl solution was prepared by replacing NaCl from the above solution with KCl. Contractions with this high KCl solution were obtained periodically throughout the experiment to assure stability of the tissues and to use as a standard maximal contraction for a given tissue. After an initial KCl contraction, successive KCl contractions were reproducible on veins when performed at 40 min intervals. After a 2.5 h stabilization period during which 3 KCl-induced contractures were produced, a cumulative concentration-effect curve for a drug was determined. Each tissue was used for only one agonist.

The physiological solution was supplemented with 6 $\mu$M cocaine to prevent neuronal uptake of both (−)-noradrenaline (Ljung *et al.*, 1973) and 5-HT (Thoa *et al.*, 1969) and to prevent the release of noradrenaline by 5-HT (Fozard *et al.*, 1979). In experiments with 5-HT, 0.1 mM ascorbate was used to retard oxidation of 5-HT.

The rhythmical mechanical activity of the portal veins was analysed by integration of force over 3 min periods with the help of an electronic digitizer coupled to an Apple computer. The affinity of ketanserin for 5-HT receptors of the portal venous system was estimated by determining 2 successive concentration-effect curves to 5-HT, the first in the absence, and the second in the presence, of ketanserin (pre-incubated for 2 h). Two successive concentration-effect curves for 5-HT in the absence of ketanserin were nearly superimposable in both superior mesenteric vein and portal vein. The nature of the antagonism of the effects of 5-HT by ketanserin was analysed by the method of Arunlakshana & Schild (1959).

### *Haemodynamic studies*

The techniques of Groszmann *et al.* (1982) were used for the haemodynamic measurements. Briefly, the animals were anaesthetized with ketamine HCl (100 mg $kg^{-1}$ i.m.). The left femoral and right carotid arteries were exposed and cannulated with PE-50 catheter. The right carotid catheter was advanced into

the left ventricle during continuous pressure monitoring. The mesenteric vein was exposed through a 2 cm midline abdominal incision and catheterized using PE-50 tubing. The catheter was fixed to the mesentery by applying cyanoacrylate glue and the abdominal wall was closed with a surgical suture. The left external jugular vein was cannulated with a PE-50 catheter which was advanced into the right atrium (during continuous pressure monitoring). All catheters were connected to Statham P-23-Db strain gauge transducers and continuous arterial and venous pressure measurements were recorded on a Grass Model 7D polygraph. For all pressure recordings the external zero references point was placed at the level of the right atrium. Rectal temperature was maintained at 37.0 ± 0.5°C by means of a heat lamp. After all the catheters had been inserted and the rat body temperature was stable, 0.3 ml of solution containing 0.3 mg kg$^{-1}$ ketanserin or 0.3 ml of 0.9% w/v NaCl solution (saline) was injected intraportally. Pressure measurements were taken before and 5 min after the injection. Flow studies were done 5 min after the treatment. Eight to nine rats were used in each of the four groups for a total of 34 animals. At the end of the experiment the animal was killed by a bolus injection of saturated KCl. The abdominal organs, kidneys, lungs and testes were dissected and weighed.

Radiolabelled microspheres were used for cardiac output and regional organ blood flow determinations using a reference sample method previously used in our laboratory (Groszmann *et al.*, 1982). The reference sample was withdrawn from the left femoral artery catheter into a preweighed syringe for 75 s at an approximate rate of 1.0 ml min$^{-1}$ using a Harvard pump (Harvard Apparatus, Millis, MA). Fifteen seconds after beginning withdrawal of the reference sample, approximately 40,000 polymeric resin, $^{141}$Ce-labelled microspheres (15 ± 3 μm diameter; New England Nuclear, Boston, MA) were injected into the left ventricle over 10–15 s. The catheter was flushed with 0.2 ml of saline.

Cardiac output (CO) and organ blood flows were calculated from the $^{141}$Ce injections:

CO (ml min$^{-1}$) = injected radioactivity (c.p.m.) × reference blood flow (ml min$^{-1}$)/reference blood radioactivity (c.p.m.)

Organ blood flow (ml min$^{-1}$) = organ radioactivity (c.p.m.) × reference blood flow (ml min$^{-1}$)/reference blood radioactivity (c.p.m.)

At least 300 microspheres were trapped in both the reference sample and organs to ensure validity of the measurement (Ishise *et al.*, 1980). A cardiac index was calculated by dividing cardiac output by body weight.

Portal venous inflow (PVI) was the sum of blood flow to stomach, spleen, small and large intestines, pancreas and mesentery. This calculation represents the total blood flow entering the portal venous system (Groszmann *et al* 1982) and was expressed in ml min$^{-1}$ 100 g$^{-1}$ body wt.

Portal-systemic shunting was quantitated using the technique described by Chojkier & Groszmann (1981). Briefly, the spleen was exposed through a small parasagital incision and intrasplenic injection of approximately 30,000 $^{103}$Ru-labelled microspheres (15 ± 3 μm) was made over 20 s. Haemostasis was achieved by cyanoacrylate glue.

Portal-systemic shunting (PSS) was calculated from the $^{103}$Ru injection as follows:

PSS(%) = lung radioactivity (c.p.m.)/(liver + lung) radioactivity (c.p.m.)

The radioactivity (c.p.m.) of each organ was determined in a gamma scintillation counter (Packard, MCA, 9015, Downers Grove, IL). Kidney and testicular radioactivity were used to check for adequate mixing of microspheres. The larger organs were dissected into smaller portions for uniform geometry within the scintillation counting tubes. The error in the measurement of the radioactivity induced by the spillover of $^{103}$Ru (energy window used at 420–650 keV) into the $^{141}$Ce channel (energy window at 50–200 keV) was corrected by using $^{103}$Ru and $^{141}$Ce standards.

Resistance in various vascular systems was calculated by the general formula:

resistance (mmHg min ml$^{-1}$ 100 g$^{-1}$ body wt.) = ΔP (mmHg)/Q(ml min$^{-1}$ 100 g$^{-1}$ body wt).

For calculating total peripheral resistance, ΔP equals mean arterial pressure minus right atrial pressure, and Q is cardiac output. The changes in right atrial pressure were less than 1 mmHg and were not considered in the calculation. Splanchnic arteriolar resistance was calculated from mean arterial minus portal venous pressure divided by PVI. For calculating portal venous resistance, ΔP is portal pressure and Q is portal venous inflow. Again the right atrial pressure was not considered in the calculation.

*Responses to intraportal drug administration*

Portal pressor responses and systemic blood pressure responses to injected drugs were studied in 10 portal hypertensive rats and 5 sham-operated rats anaesthetized with ketamine. The femoral artery and mesenteric vein were catheterized as described previously and arterial and portal pressures measured continuously. Intraportal injections of 5-HT and (−)-noradrenaline were carried out in 50 or 100 μl of saline followed by a flush with 100 μl saline. Responses to an amine dose were recorded until the pressures had returned to baseline values for at least 5 min before the

administration of another dose. Responses to both 5-HT and (−)-noradrenaline were repeated after the administration of 0.3 mg kg$^{-1}$ ketanserin.

*Plasma catecholamines*

Noradrenaline and adrenaline were determined by a radioenzymatic assay (catechol-*O*-methyl transferase; CAT-A-KIT assay kit, Upjohn, Kalamazoo, Michigan). The femoral artery was catheterized as described above in 6 portal hypertensive and 6 sham-operated rats under ketamine anaesthesia. A sample, 2 ml of blood was withdrawn into a tube containing EGTA and glutathione.

*Drugs*

5-Hydroxytryptamine hydrochloride was obtained from Sigma (St. Louis, MO) and dissolved in 0.1 mM ascorbic acid. Ketanserin tartrate was obtained from Janssen (Neuss, FRG). (−)-Noradrenaline (−)-tartrate was obtained from Merck-Schuchardt (Hohenbrun b. München, FRG). Vasopressin and ketamine were from Parke Davis (Morris Plains, NJ, U.S.A.).

*Statistics*

Results are expressed as means ± s.e.mean. The significance of differences was assessed by means of Student's *t* test at a *P* level of $<0.05$.

## Results

*Reactivity of isolated superior mesenteric veins to vasoconstrictors*

Unlike portal veins which showed spontaneous contractile activity, superior mesenteric veins were quiescent. 5-HT, (−)-noradrenaline, vasopressin and KCl caused tonic contractions of the superior mesenteric veins. In agreement with previous results (Groszmann & Kaumann, 1983) it was found that the superior mesenteric veins of portal hypertensive rats exhibited and enhanced response to 5-HT and a decreased response to (−)-noradrenaline (Figure 1) compared with sham-operated rats. The response to 128 mM KCl was 0.97 ± 0.22 mN in 11 veins from sham-operated rats and 1.60 ± 0.51 mN ($P = 0.18$) in 7 veins from portal hypertensive rats. Concentration-effect curves for 5-HT were shifted to the left while the curves for (−)-noradrenaline were shifted to the right in veins from portal hypertensive rats compared to veins from sham-operated rats (Figure 1). As with 5-HT, the maximum response to vasopressin was increased 3 fold in veins from portal hypertensive rats; however,

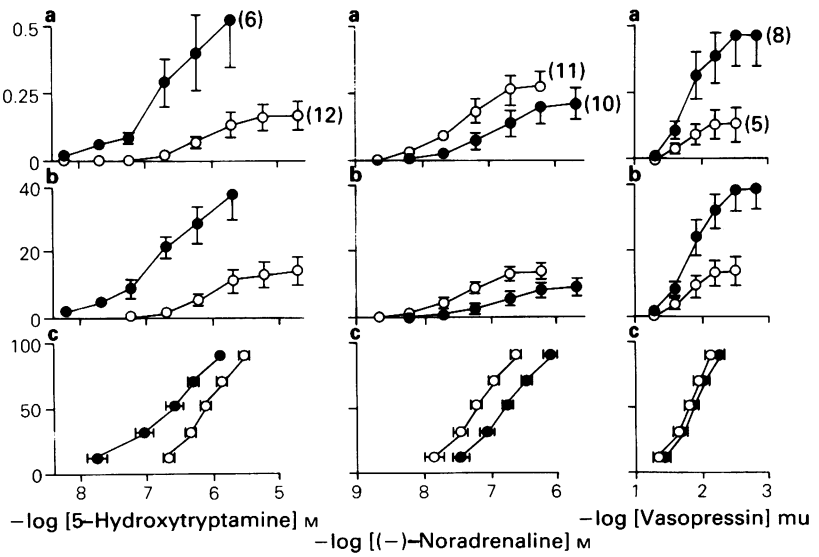


**Figure 1** A comparison of the reactivity to vasoactive agents of superior mesenteric veins from sham-operated (○) and portal vein ligated (●) rats. The rats were operated upon 14 days before the experiments were carried out. A single concentration-effect curve was determined for each drug on each vein. Reproducible contractures to 128 mM KCl were determined before drug administration and after washout of the drug. The contractile responses to the drugs are expressed as increases in force (ΔmN, a), as % of the contracture to 128 mM KCl (b) and as % of the maximum effect to each drug (c). The concentration of vasopressin refers to mu per 50 ml. The number of veins is shown in parentheses in (a).

concentration-effect curves for vasopressin were not shifted in veins from portal hypertensive rats compared to veins from sham-operated rats (Figure 1).

Superior mesenteric veins of portal hypertensive rats weighed 3 times more than veins from sham-operated rats ($6.0 \pm 1.3$ mg cm$^{-1}$, $n = 15$, vs $1.7 \pm 0.1$ mg cm$^{-1}$, $n = 8$).

*Antagonism by ketanserin of the effects of 5-HT in isolated veins*

Ketanserin (0.6–200 nM) did not affect contractions caused by 128 mM KCl in either portal or mesenteric veins, and did not influence the rhythmic contractions of portal veins (not shown). Ketanserin caused surmountable antagonism of the effects of 5-HT in both superior mesenteric and portal veins. Concentration-effect curves for 5-HT were shifted to the right in a nearly parallel manner in the presence of ketanserin in both superior mesenteric and portal veins.

The slope of Schild plots was not different from 1, regardless of tissue or condition (Figure 2), as expected for unimolecular competition of ketanserin and 5-HT for a receptor. Ketanserin exhibited the same high affinity for the 5-HT receptors of superior mesenteric vein and portal vein, both in the sham-operated and portal hypertensive rats (Table 1).

*Normal arterial reactivity to (−)-noradrenaline*

Concentration-effect curves for contractile effects of (−)-noradrenaline on helicoids of aorta and mesenteric artery were not different in tissues from portal hypertensive and sham-operated rats. The absolute maximum effects of (−)-noradrenaline in aortae and mesenteric arteries were not different in the 2 groups of

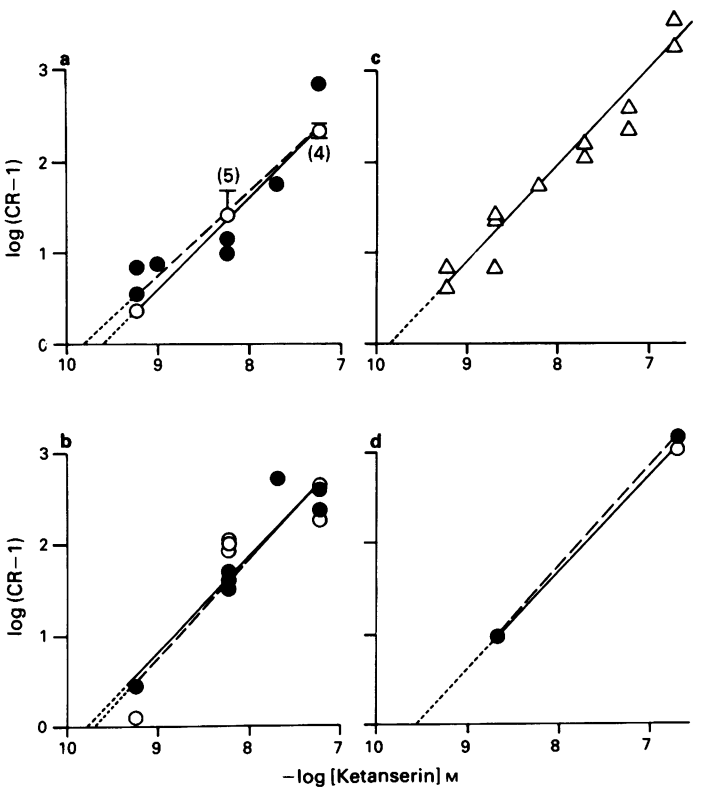


**Figure 2** Schild-plots for ketanserin as antagonist of the effects of 5-hydroxytryptamine (5-HT). Superior mesenteric veins (a) and portal veins (b) from sham-operated (○, solid lines) and portal vein ligated (●, broken lines) rats operated upon 14 days before the experiments were carried out. (c) Portal veins from normal (△) rats. (d) Portal veins from sham-operated (○, solid lines) and portal vein ligated (●, broken lines) rats, operated upon 2 days before the experiments were carried out. Each symbol is the result from one vein, except the 2 open circles with bars in (a) which represent mean ± s.e.mean of the number of veins shown in parentheses. For slopes of Schild plots and $K_B$ values see Table 1.

**Table 1** Antagonism of 5-hydroxytryptamine (5-HT)-induced contractions by ketanserin

| *Tissue* | *Condition* | n | *Slope of Schild plot* | $-\log K_{B(M)}$ (mean ± s.e.mean) |
|---|---|---|---|---|
| SMV | Sham 14 days | 10 | 0.97 | 9.59 ± 0.13 |
| SMV | PH 14 days | 6 | 0.91 | 9.69 ± 0.16 |
| Portal vein | Sham 14 days | 6 | 1.05 | 9.88 ± 0.17 |
| Portal vein | PH 14 days | 7 | 1.08 | 9.99 ± 0.24 |
| Portal vein | Not operated | 14 | 1.06 | 9.95 ± 0.08 |
| Portal vein | Sham 2 days | 2 | 1.04 | 9.72 |
| Portal vein | PH 2 days | 2 | 1.10 | 9.78 |

Abbreviations used: SMV, superior mesenteric vein; PH, portal hypertensive; *n*, number of tissues. $K_B$ (equilibrium dissociation constant) [B]/(CR − 1), where [B] is the concentration of ketanserin used, and CR the concentration-ratio of 5-HT measured at 50% of maximum effect.

rats. The $EC_{50}$ values (− log M) for (−)-noradrenaline were 8.2 ± 0.2 and 8.3 ± 0.3 for the mesenteric artery and 8.6 ± 0.3 and 8.8 ± 0.2 for the aorta from sham-operated ($n = 4$) and portal hypertensive rats ($n = 4$), respectively.

*Selective blockade by ketanserin of the pressor effects of 5-HT*

The intraportal administration of 5-HT caused an inconstant initial small rise in portal pressure usually followed (but not always, Figure 3) by a decrease in blood pressure along with a marked dose-dependent increase in portal pressure. This was seen in both sham-operated and portal hypertensive rats. For 26% of the doses of 5-HT in the portal hypertensive rats, and 22% of the doses of 5-HT in the sham-operated rats, an initial arterial pressor response (Figure 3) was seen. This effect was not dose-dependent. The concentration of 5-HT that caused a threshold increase in portal pressure was significantly lower in portal hypertensive rats than in sham-operated rats (Figure 4). With the exceptions mentioned above most rats exhibited secondary decreases of blood pressure with increasing doses of 5-HT (Figure 4).

The intraportal administration of (−)-noradrenaline caused an initial small pressor response in the portal vein followed by an increase in systemic blood pressure and a more pronounced and dose-dependent increase in portal pressure (Figures 3 and 4). The initial portal pressor response to 150 ng (−)-noradrenaline was 0.52 ± 0.08 mmHg in 5 sham-operated rats and 0.17 ± 0.09 mmHg ($P < 0.02$) in 7 portal hypertensive rats.

The intraportal injection of 0.03–0.3 mg kg$^{-1}$ ketanserin significantly decreased portal pressure in portal hypertensive rats (Figure 3) but did not in sham-operated rats. Ketanserin 0.3 mg kg$^{-1}$ did not affect the portal and systemic pressor responses to (−)-noradrenaline but reduced the portal pressor responses to 5-HT in both groups of rats (Figure 4). The inconstant systemic pressor effects of 5-HT were reversed into depressor responses by 0.3 mg kg$^{-1}$ ketanserin (Figure 3). The systemic depressor respon-

**Table 2** Pressure and heart rate measurement before and after ketanserin or saline administration

| | *Mean arterial pressure* (mmHg) | | *Portal pressure* (mmHg) | | *Right atrial pressure* (mmHg) | | *Heart rate* (beats min$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | *Baseline* | *Final* | *Baseline* | *Final* | *Baseline* | *Final* | *Baseline* | *Final* |
| Portal hypertensive saline ($n = 8$) | 107 ± 6 | 104 ± 6 | 13.5 ± 0.8 | 13.8 ± 0.8 | 0.3 ± 0.1 | 0.2 ± 0.2 | 337 ± 17 | 343 ± 17 |
| Portal hypertensive ketanserin ($n = 9$) | 113 ± 6 | 92 ± 5* | 13.7 ± 0.6 | 11.3 ± 0.4* | − 0.1 ± 0.3 | − 0.6 ± 0.3 | 330 ± 15 | 304 ± 13* |
| Sham-operated saline ($n = 8$) | 123 ± 1 | 121 ± 2 | 8.1 ± 0.2 | 8.1 ± 0.2 | 0.2 ± 0.2 | 0.2 ± 0.2 | 329 ± 8 | 328 ± 8 |
| Sham-operated ketanserin ($n = 9$) | 121 ± 7 | 104 ± 6* | 7.6 ± 0.1 | 7.5 ± 0.3 | 0.7 ± 0.2 | 0.5 ± 0.2 | 347 ± 15 | 328 ± 17* |

Final values were obtained 5 min after the administration of saline or 0.3 mg kg$^{-1}$ ketanserin. *$P < 0.05$ compared to baseline (paired *t* test).

ses to 5-HT appeared somewhat more pronounced after 0.3 mg kg$^{-1}$ ketanserin but this was only significant with high concentrations of 5-HT in the portal hypertensive group (Figure 4).

*Haemodynamic effects of ketanserin*

Baseline measurements including arterial pressure, portal venous pressure, right atrial pressure and heart rate were similar in all groups. Average weight at the time of the study was 340 ± 1 g. Table 2 shows the pressure and heart rate values at baseline and after drug (ketanserin or saline) administration.

In the portal hypertensive rats intraportal administration of 0.3 mg kg$^{-1}$ ketanserin produced a significant decrease in blood pressure along with a

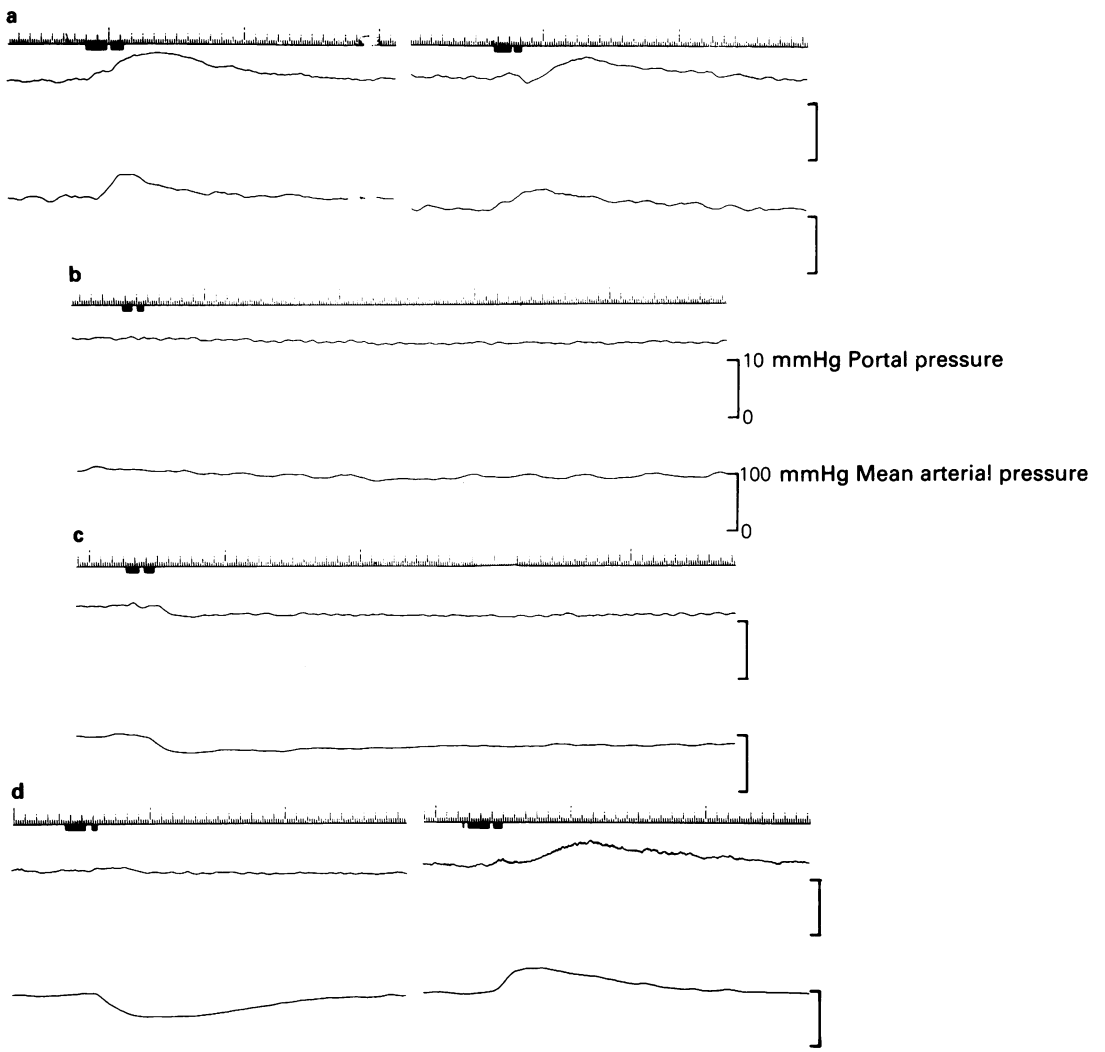


**Figure 3** Selective antagonism by ketanserin of the portal and arterial pressor responses to 5-hydroxytryptamine (5-HT) in a portal hypertensive rat. Intraportal administration of 5-HT, (−)-noradrenaline and ketanserin. Intervals between largest time marker indicate 1 min. In all panels the top trace corresponds to portal pressure and the bottom trace to systemic arterial pressure. The lefthand traces of (a) and (d) depict the response to 10 μg 5-HT. The righthand traces of (a) and (d) depict the response to 150 ng (−)-noradrenaline. (b) and (c) Show the effect of the administration of 10 μg and 90 μg ketanserin, respectively. Injections of drugs were made during the first marker in a volume of 100 μl, followed by a 100 μl flush with saline indicated by the second marker.

significant decrease in portal pressure. An 18% decrease in mean arterial pressure and an 18% decrease in portal pressure were noted when post-injection measurements were compared to their pre-injection baseline values. No changes in mean arterial pressure or portal pressure were noted in the saline-treated portal hypertensive group (Table 2).

In the sham-operated rats given ketanserin a significant, 14%, reduction in blood pressure was noted but there was no change in portal pressure. In the saline-treated sham group no pressure changes were noted. A significant reduction in heart rate was noted in both portal hypertensive and sham-operated rats given ketanserin (8% and 6% respectively, $P < 0.05$). No changes were noted in right atrial pressures (Table 2). Blood flow measurements were not available for baseline *vs* post-ketanserin or saline comparison. Blood flow using the radioactive microsphere tech-

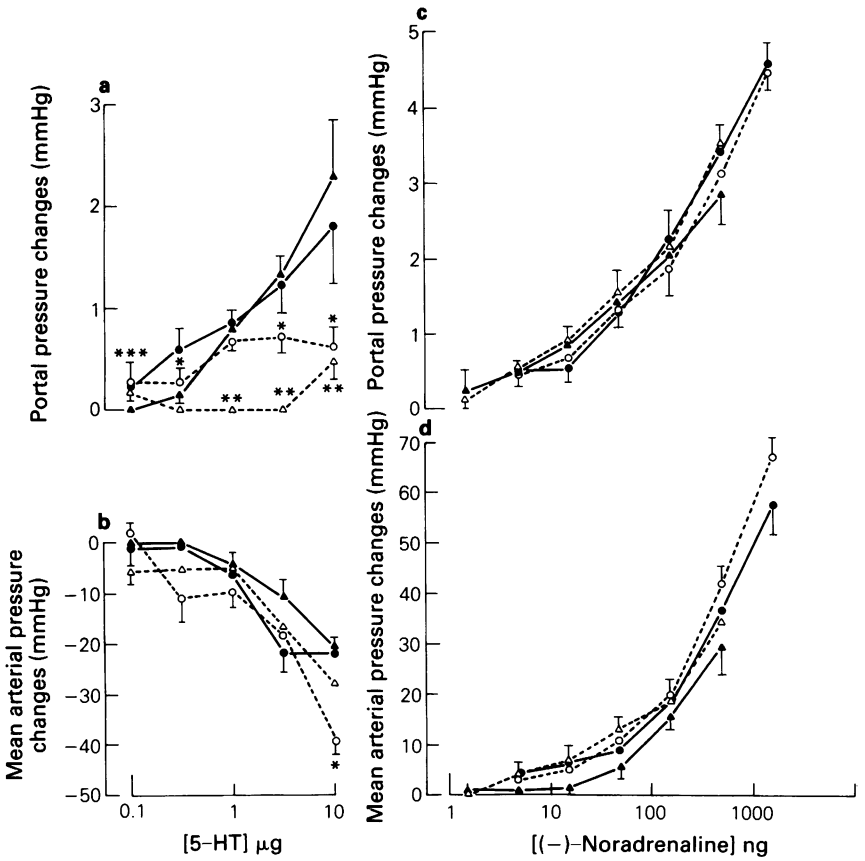


**Figure 4** Effects of 5-hydroxytryptamine (5-HT) and (−)-noradrenaline on arterial and portal pressure before and after 0.3 mg kg$^{-1}$ ketanserin blockade in portal hypertensive and sham-operated rats. (a) Shows the change in portal pressure and (b) in mean arterial pressure after 5-HT administration. (c) Shows the change in portal pressure and (d) in mean arterial pressure after noradrenaline administration. (●——●) Represents results from portal hypertensive rats; (○ - - - ○), portal hypertensive rats after ketanserin; (▲——▲), sham-operated rats; (△ - - - △), sham-operated rats after ketanserin. Each point represents the mean of 10 experiments in the portal hypertensive group and 5 experiments in the sham-operated group; vertical lines show s.e.mean (some s.e.mean were excluded for clarity). Baseline measurements (mmHg) at the start of the experiment, just before ketanserin administration and after stabilization of the ketanserin effect, were: 121 ± 5, 116 ± 4, 102 ± 5 for mean arterial pressure in the portal hypertensive group; 14.7 ± 0.4, 14.6 ± 0.6, 12.7 ± 0.7 for portal pressure in the portal hypertensive group; 121 ± 7, 109 ± 9, 84 ± 6 for mean arterial pressure in the sham-operated group; 7.2 ± 0.2, 7.6 ± 0.4, 6.7 ± 0.2 for portal pressure in the sham-operated group. *$P < 0.05$, control portal hypertensive rats (●) vs those administered ketanserin (○); **$P < 0.05$, control sham-operated rats (▲) vs those administered ketanserin (△); ***$P < 0.05$, control portal hypertensive rats (●) vs control sham-operated rats (▲).

nique was measured only once in each animal. More than one systemic injection of microspheres may produce alterations in splanchnic haemodynamics (Bonaccorsi *et al.*, 1978).

Portal hypertensive and sham-operated rats receiving ketanserin were compared to their respective controls which received an equivalent volume of saline, the results are presented in Figure 5. The portal hypertensive rats treated with ketanserin exhibited a significantly lower portal pressure, cardiac output and portal flow than the portal hypertensive rats treated with saline (Figure 5). In the ketanserin-treated sham-operated rats the only significant observation was a reduced systemic blood pressure when compared to the sham-operated animals treated with saline (Figure 5). The calculated portal-collateral and splanchnic arterial resistance were not changed by ketanserin in the 2 groups (Figure 5).

Increases in bronchial flow were noted after ketanserin administration in both portal hypertensive and sham-operated rats (1.10 ± 0.20 to 3.01 ± 0.48 and 0.67 ± 0.18 to 2.60 ± 0.04 ml min$^{-1}$ 100 g$^{-1}$ body wt., respectively). The cause of this effect is unknown. Portal-systemic shunting was greater than 90% in the portal hypertensive rats and was not altered by ketanserin administration.

*Normal plasma catecholamines*

Catecholamine levels were (pg ml$^{-1}$), 626 ± 84 and 546 ± 54 for noradrenaline and 346 ± 35 and 323 ± 29 for adrenaline in 6 sham-operated rats and 6 portal hypertensive rats, respectively.

## Discussion

*Hyposensitivity to (−)-noradrenaline*

In agreement with our previous results (Groszmann & Kaumann, 1983) we found that the smooth muscle of superior mesenteric veins of portal hypertensive rats is less sensitive to (−)-noradrenaline than that of veins from sham-operated rats. The attenuation of the responses to (−)-noradrenaline could be caused by desensitization due to an enhanced sympatho-adrenal

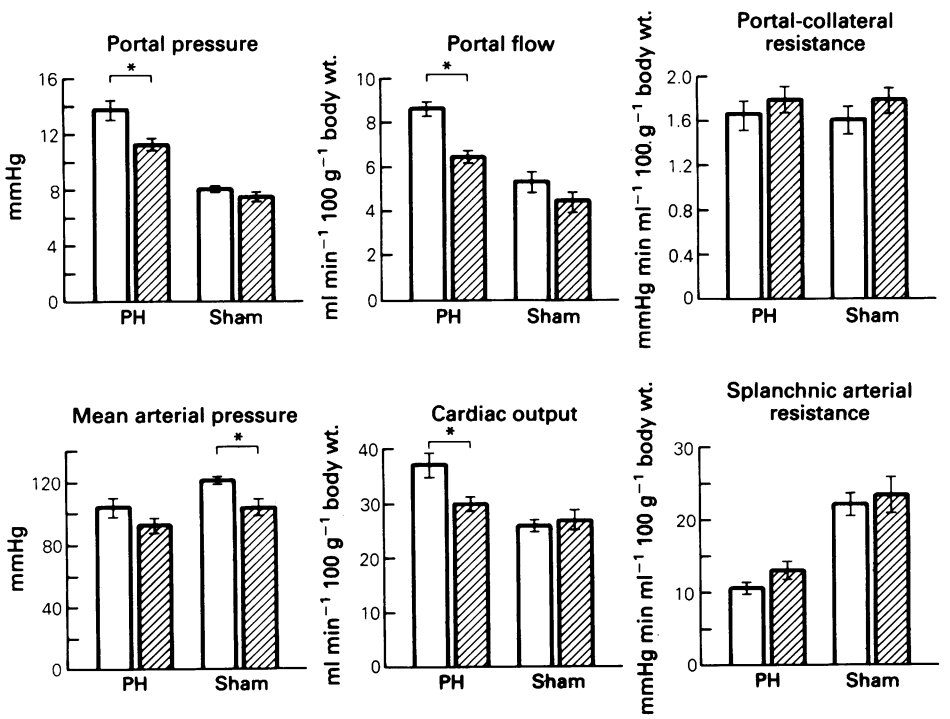


**Figure 5** A comparison between the effects of ketanserin (100 µl containing 0.3 mg kg$^{-1}$ hatched columns and saline 100µl, open columns) on splanchnic and systemic haemodynamics in portal hypertensive (PH) and sham-operated (sham) rats. Each column represents the mean of 8 (sham-operated) or 9 (portal hypertensive) rats and vertical lines indicate s.e.mean. *$P < 0.01$, portal hypertensive vs sham-operated rats (unpaired $t$ test).

activity. Clinical evidence suggests that cirrhotic patients with portal hypertension have increased plasma levels of (−)-noradrenaline (Bichet *et al.*, 1982; Ring-Larsen *et al.*, 1982) secondary to its increased secretion rather than to decreased metabolic clearance (Nicholls *et al.*, 1985). It is possible that these patients could exhibit down-regulation of their α-adrenoceptor population because of the exposure to elevated plasma catecholamines. However, for several reasons it is unlikely that desensitization to catecholamines in portal hypertensive rats is an important factor causing decreased effects of (−)-noradrenaline. Firstly, plasma catecholamines levels were not different in portal hypertensive rats and sham-operated rats 14 days after surgery. Secondly, the sensitivity of both aorta and mesenteric artery to (−)-noradrenaline was not different in portal hypertensive rats and sham-operated rats. Thirdly, β-adrenoceptor-mediated relaxation of potassium-precontracted mesenteric veins appeared to be similar in portal hypertensive and sham-operated rats (Groszmann & Kaumann, 1983).

The small initial pressor response to intraportally administered (−)-noradrenaline is probably due to a direct constriction of the venous vessels of the portal vascular bed. The attenuation of this response in portal hypertensive rats compared to sham-operated rats is consistent with the hyposensitivity to (−)-noradrenaline observed in isolated superior mesenteric veins. However, the late pressor response of portal veins appears to be the result, at least in part, of the spill-over of (−)-noradrenaline into the systemic circulation and the resultant increase in systemic blood pressure. Although the ultimate reasons for the attenuation of the portal venoconstrictor response to (−)-noradrenaline in portal hypertensive rats needs elucidation, our findings do not support an important involvement of noradrenaline in the increased portal-collateral resistance observed in this model of portal hypertension.

*Supersensitivity to 5-HT*

Superior mesenteric veins of portal hypertensive rats exhibited a 3 fold increase in the maximum response to 5-HT and vasopressin but only a marginal increase in contractions caused by 128 mM KCl compared to veins from sham-operated rats. This pattern of vascular reactivity cannot be explained consistently by a 3 fold increase in the mass of smooth muscle encountered by us in veins from portal hypertensive rats with respect to that of veins from sham-operated rats. A discrepancy between hypertrophied smooth muscle of portal veins and lack of a proportional increase of contractile responses to noradrenaline was previously observed by Johansson (1976). Our data suggest that the KCl contraction and contractions induced by both 5-HT and vasopressin are affected differently by portal hypertension. Clearly, the availability of calcium for contraction induced by the two drugs must differ from the amount of calcium flowing into the smooth muscle cells during 128 mM KCl-induced depolarization.

The hypertrophied smooth muscle of superior mesenteric veins of portal hypertensive rats was sensitized to 5-HT but not to vasopressin. Reasons for this selective sensitization are unknown. The sensitization to 5-HT is not accompanied by an increased affinity of ketanserin for 5-$HT_2$-receptors.

When the portal pressure response to 5-HT was compared in portal hypertensive and sham-operated rats, the sensitization to 5-HT only became manifest at low concentrations. This agrees only partially with the results obtained on isolated superior mesenteric veins. The quantitative discrepancy between the *in vitro* and *in vivo* experiments is probably related to limitations of the *in vivo* preparation. The spill-over of 5-HT into the systemic circulation and the appearance of systemic effects probably hampers the precise appraisal of direct effects of 5-HT on the intact portal venous system.

*The portal venous system appears to possess 5-$HT_2$-receptors*

The competitive pattern of antagonism of the effects of 5-HT by ketanserin suggests involvement of a single class of 5-HT receptors in the portal venous system of the rat. The affinity of ketanserin (i.e. $K_B$ values of 0.1–0.3 nM) estimated for the smooth muscle of both superior mesenteric veins and portal veins agrees with similar affinity estimates for ketanserin on arterial smooth muscle (Kaumann, 1983; Frenken & Kaumann, 1984; Kaumann & Frenken, 1985) and rat brain (Leysen *et al.*, 1982). This agreement suggests that the 5-HT receptors of the portal vascular system are 5-$HT_2$-receptors.

The involvement of 5-$HT_2$-receptors in the portal venous system is also suggested by the finding that ketanserin blocked the portal pressor effects of 5-HT in both sham-operated and portal hypertensive rats. The antagonism by ketanserin of the portal pressor responses to 5-HT was evident in all rats, regardless of whether 5-HT caused pressor or depressor effects in the systemic circulation. This evidence is consistent with the assumption that the portal venoconstrictor effects are elicited through a direct interaction of 5-HT with 5-$HT_2$-receptors located in the portal venous system.

Ketanserin tended to enhance the depressant effects of 5-HT on systemic arterial pressure or reverse the pressor response to 5-HT to a depressor response. It is well known that 5-HT can cause both increases and decreases in blood pressure. The increases in pressure

are due, at least in part, to an interaction of 5-HT with arterial 5-HT$_2$-receptors while the decreases in pressure may result from the release of a vasodilator factor derived from endothelial cells (Cohen *et al.*, 1983). By blocking 5-HT$_2$-receptors in our experiment ketanserin probably unmasked or accentuated the arterial dilatory effects of 5-HT.

*Ketanserin and α-adrenoceptors*

Although 0.3 mg kg$^{-1}$ ketanserin antagonized the portal pressor responses to 5-HT it did not affect the portal and systemic pressor responses to (−)-noradrenaline. Similar observations were made by Fozard (1982) and Persson *et al.* (1982) with ketanserin on rat arterial pressure. Since 0.3 mg kg$^{-1}$ ketanserin does not block the effects of exogeneous (−)-noradrenaline it is unlikely that circulating noradrenaline is involved in the portal hypotensive effect of ketanserin in the rat. Ketanserin 0.25 mg kg$^{-1}$ antagonizes pressor responses elicited by sympathetic stimulation through $\alpha_1$-adrenoceptors (Fozard, 1982). α-Adrenoceptors distinct from $\alpha_1$-receptors may participate to varying degrees in the arterial constrictor responses to (−)-noradrenaline in various vascular regions (Drew & Whiting, 1979). The nature of the α-adrenoceptor subtypes involved in portal pressor responses to circulating noradrenaline and to sympathetic nerve stimulation still needs to be elucidated.

*Haemodynamics of ketanserin in portal hypertension*

After ketanserin administration, a reduction in cardiac output was observed only in portal hypertensive animals. The reason for this selective effect in the portal hypertensive animals may be related to the role played by 5-HT. We propose that 5-HT plays an important role in maintaining the venous tone in these animals. Normally catecholamines help maintain portal venous tone (Greenway, 1983) but, as shown in this study, this effect may be impaired in portal hypertension. Therefore, the dual defect of both hyporesponsiveness to circulating catecholamines and hyperresponsiveness to 5-HT may lead to marked portal venous pooling in the face of blockade of the effects of 5-HT by ketanserin. It is also conceivable that the $\alpha_1$-adrenoceptor blocking properties of ketanserin may contribute to the above described mechanism. By decreasing venous tone and therefore the venous return to the heart, ketanserin causes a decrease in cardiac output. The reduction in cardiac output could explain the decrease in portal venous inflow in the absence of a significant increase in splanchnic arterial resistance.

The portal pressure lowering effect of ketanserin was specific to the portal hypertensive rats and was absent in the sham-operated rats, suggesting a specific effect of ketanserin in portal hypertension. The decrease in portal pressure was mainly due to a decrease in portal venous inflow. Although the calculated portal-collateral resistance in portal hypertensive rats is not different from that in sham-operated rats, it is inappropriately high for the elevated portal venous inflow observed in these animals. When, in normal rats, the portal venous inflow is increased to values comparable to portal hypertensive blood flow rates the portal-collateral resistance is reduced to values significantly lower than those observed in portal hypertensive rats. Thus, portal pressure stays within normal limits (Kroeger & Groszmann, 1985a). This can be best explained by noting that when blood flow increases in normal blood vessels, resistance falls owing to further distension of the vessels (Selkurt, 1976). In the portal hypertensive rat, for every portal blood flow rate, high or low, the altered network of collateral vessels imposes a higher resistance level, and thus a higher resistance flow product ($\Delta P = R \times F$), than the one observed in the vascular pathway of the sham-operated rat at equal flows. At every flow rate the portal hypertensive animal has a higher portal pressure than the sham-operated rat (Kroeger & Groszmann, 1985a; Sikuler & Groszmann, 1986). Ketanserin, unlike β-adrenoceptor blocking agents and nitroglycerin, did not produce an increase in portal collateral resistance during portal blood flow reduction (Kroeger & Groszmann, 1985a, b). Therefore, the decrease in portal flow induced by ketanserin, unopposed by an increase in portal-collateral resistance, caused a larger decrease in portal pressure than seen with other agents that reduce portal flow to equivalent levels.

The decrease in portal venous inflow induced by ketanserin was not accompanied by an increase in portal-collateral resistance, a detrimental phenomenon observed with other agents. Ketanserin should be explored as a potentially useful agent in the treatment of portal hypertension.

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