5-HT\textsubscript{1}-like receptor mediated changes in porcine carotid haemodynamics: are 5-HT\textsubscript{1D} receptors involved?

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Summary. 5-Hydroxytryptamine (5-HT) reduces porcine arteriovenous shunting in the carotid vascular bed by stimulation of both 5-HT\textsubscript{1}-like and 5-HT\textsubscript{2} receptors and increases capillary flow to some tissues, like the skin and ears, by different 5-HT\textsubscript{1}-like receptors. In view of the heterogeneous nature of the 5-HT\textsubscript{1}-like receptors and the relative selectivity for the 5-HT\textsubscript{1D} binding sites of sumatriptan, which also reduces porcine arteriovenous shunting and slightly increases capillary blood flow towards skin and ears by 5-HT\textsubscript{1}-like receptors, we have attempted to determine whether one or both of these carotid 5-HT\textsubscript{1}-like receptors belong to the 5-HT\textsubscript{1D} subtype.

Pentobarbitone anaesthetized pigs, subjected to bilateral cervical vagosympathectomy, received either 5-HT (2 \(\mu\)-g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\)) in the carotid artery or cumulative i.v. doses of sumatriptan (10, 30, 100 and 300 \(\mu\)-g \(\cdot\) kg\(^{-1}\)). Their effect on the total carotid blood flow and its distribution into capillary and arteriovenous anastomotic parts was determined with radioactive microspheres. The effect of metergoline (1 mg \(\cdot\) kg\(^{-1}\)), a substance with a very high affinity for the 5-HT\textsubscript{1D} receptor as well as for the 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors, was studied on the responses to 5-HT and sumatriptan.

Both 5-HT and sumatriptan reduced carotid arteriovenous anastomotic blood flow, 5-HT and, to a lesser extent, sumatriptan also increased capillary blood flow towards some tissues. Metergoline by itself did not affect the distribution of porcine carotid blood flow. It attenuated the constrictor response, but increased the vasodilator response to 5-HT, in a manner similar to the 5-HT\textsubscript{2} receptor antagonists cyproheptadine, ketanserin and WAL 1307 in our former experiments. These effects seem, therefore, to be related to the blockade of 5-HT\textsubscript{2} receptors by metergoline. On the other hand, metergoline had no significant effect against the responses to sumatriptan.

It is concluded that neither the constrictor nor the dilator carotid 5-HT\textsubscript{1}-like receptors seem to be related to the known 5-HT\textsubscript{1} binding subtypes, including the 5-HT\textsubscript{1D} subtype.

Key words: Arteriovenous anastomoses – Metergoline – Sumatriptan – 5-HT – 5-HT receptors

Introduction

Both 5-hydroxytryptamine (5-HT) and sumatriptan decrease arteriovenous shunting of carotid blood flow in anaesthetized animals, including the domestic pig. This decrease in arteriovenous shunting by 5-HT is mediated by both 5-HT\textsubscript{1}-like and 5-HT\textsubscript{2} receptors, since this effect is abolished by methiothepin (Saxena et al. 1986) and reduced by cyproheptadine (Saxena and Verdouw 1982) and ketanserin (Verdouw et al. 1984). In the case of sumatriptan, constriction of arteriovenous anastomoses (Perren et al. 1989; Den Boer et al. 1991) is mediated exclusively via 5-HT\textsubscript{1}-like receptors, since methiothepin, but not ketanserin, acts as an antagonist (Den Boer et al. 1991). Furthermore, 5-HT and, to a lesser extent, sumatriptan increase capillary flow towards some tissues, like ears and skin; this effect is mediated by 5-HT\textsubscript{1}-like receptors, since methiothepin, but not ketanserin, appears to be a 5-HT\textsubscript{1}-like receptor agonist which also decreases arteriovenous shunting, does not increase skin or ear blood flow (Villalón et al. 1990).

Within the 5-HT\textsubscript{1} receptor family sumatriptan has a relative selectivity for the 5-HT\textsubscript{1D} receptors (Peroutka and McCarthy 1989; Schoeffter and Hoyer 1990), which have been defined in the brain of various species, including the domestic pig (Waebler et al. 1988). The 5-HT\textsubscript{1D} receptor has received much interest lately because several actions of 5-HT and/or sumatriptan, for example, endothelium-dependent coronary vasodilatation in the pig...
After an overnight fast 20 domestic pigs (Yorkshire×Landrace; i.v. infusion of pentobarbitone sodium (Sanofi, Paris, France) at PO2:100-120 mmHg). Anaesthesia was maintained with a continuous
vection within physiological limits (pH: 7.35-7.48; pCO2:35-48 mmHg;
volume and oxygen supply were adjusted to keep arterial blood gas val-
ulation with a mixture of room air and oxygen. Respiratory rate, tidal

Methods

the substances that have currently been tested, meterg-
to the 5-HT1D subtype. Therefore, we studied the carotid
putative 5-HT1D receptor agonist sumatriptan in the ab-
ceptors in the pig carotid circulation, especially with the
nature of the constrictor and dilator 5-HT2-like re-
mediating the reduction in porcine carotid arteriovenous
anastomotic blood flow by indorenate is not related to
any of the subtypes of 5-HT binding sites, including the
5-HT1D (Villal6n et al. 1990).

In the present experiments, we have further explored the
nature of the constrictor and dilator 5-HT1-like rece-
ceptors in the pig carotid circulation, especially with the
aim of verifying if one or both of these receptors belong to the
5-HT1D subtype. Therefore, we studied the carotid
vascular effects of the autogenous ligand 5-HT, and the
putative 5-HT1D receptor agonist sumatriptan in the ab-
ence or the presence of metergoline (1 mg·kg-1). Of all
the substances that have currently been tested, metergo-
line possesses the highest affinity for the 5-HT1D recep-
tors (Waeber et al. 1988).

General

After an overnight fast 20 domestic pigs (Yorkshire×Landrace; 16–22 kg) were anaesthetized with azaperone (120 mg, i.m.) and meto-
midate (150 mg, i.v.), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure venti-
lation with a mixture of room air and oxygen. Respiratory rate, tidal
volume and oxygen supply were adjusted to keep arterial blood gas val-
ues within physiological limits (pH: 7.35–7.48; pCO2: 35–48 mmHg; PO2: 100–120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbione sodium (Sanofi, Paris, France) at
20 mg·kg-1·h-1 for the first hour and thereafter 12 mg·kg-1·h-1.

Catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery,
connected to a Statham pressure transducer (P23 DC, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure and the with-
drawal of arterial blood for determining blood gases (ABL-2, Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was cal-
culated from the systolic (SAP) and diastolic (DAP) arterial pressures: MAP = (SAP + 2 × DAP)/3. The common carotid arteries were dissec-
ted free and the cervical vagosympathetic trunks were cut. Blood flow
was measured in one of the common carotid arteries with a flow probe
(internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic
flow meter (Transflow 600-system, Skalar, Delft, The Nether-
lands). Heart rate was measured with a tachograph triggered from the
blood pressure or the flow signal, depending on their shape. A 0.5 mm
(external diameter) needle, connected to a polyethylene tubing was in-
serted into the common carotid artery against the direction of the blood
flow for the administration of radioactive microspheres. In the animals
that were given 5-HT a second needle was inserted into the same carotid
artery for the infusion of 5-HT. At the same side the jugular vein was
 cannulated in order to obtain venous blood samples for determining
blood gases.

During the experiment body temperature was kept at about 37 °C and the animals were continuously infused with 100 ml·h-1 saline to
compensate for fluid losses.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with
15·1 (SD) mm diameter microspheres labelled with either 141Ce, 113Sn, 106Ru, 39Nb or 46Sc (REN Company, Dreieich, FRG). For each measure-
ment a suspension of about 20000 microspheres, labelled with one of
the, randomly assigned, isotopes, was mixed and injected into the
carotid artery against the direction of the blood flow to ensure uniform
mixing. At the end of the experiment the animals were killed and the
heart, kidneys, lungs and the different cranial tissues were dissected out,
weighed and put in vials. The radioactivity in these vials was counted for 5–10 min in a gamma-scintillation counter (Packard, Minaxi Auto-
gamma 5000) using suitable windows for discriminating the different
isotopes.

The ratio between the radioactivity in a particular tissue and the to-
tal radioactivity was calculated with a set of specially developed com-
puter programs (Saxena et al. 1980). By multiplying this ratio with the
total carotid blood flow value at the time of the injection, blood flow to
the tissues (capillary blood flow) was determined. No radioactivity
could be detected in the heart or the kidneys, so all microspheres reach-
ing the venous side by arteriovenous anastomoses were trapped in the
lungs. Therefore, the amount of radioactivity in the lungs was used as
an index for the arteriovenous anastomotic part of the common carotid
blood flow (see Johnston and Saxena 1978; Saxena and Verdouw 1984).

Experimental protocols

5-HT experiments. The experiments were started after a stabilization
period of about 1 h. After measuring heart rate, mean arterial blood
pressure, carotid blood flow and its distribution and arterial and jugular
venous blood gases at baseline, an intracarotid infusion of 5-HT
(2 µg·kg-1·min-1), lasting about 15 min, was started. All parameters
were reassessed towards the end of this infusion and, again, 30 min after
terminating the infusion (recovery). Then, metergoline (1 mg·kg-1) was
administered i.v. over a period of 30 min. All parameters were again
assessed before and during a second 5-HT infusion (same protocol as
above).

Sumatriptan experiments. After a stabilization period of about 1 h, the
animals were divided into two groups. The first group received cumula-
tive i.v. doses of sumatriptan (10, 30, 100 and 300 µg·kg-1·min-1) every
20 min after saline pretreatment (n = 8). The second group received the
same doses of sumatriptan after pretreatment with metergoline
(1 mg·kg-1), administered over a 30 min period (n = 7). Just before
and 15 min after each dose of sumatriptan measurements of heart rate,
mean blood pressure, carotid blood flow and its distribution and arteri-
al and jugular venous blood gases were made.

Data presentation and statistical analysis

All data have been expressed as mean±SEM. The significance of the
differences between the variables within one group was evaluated with
Duncan's new multiple range test, once an analysis of variance (ran-
domized block design) had revealed that the samples represented differ-
ent populations (Steel and Torrie 1980). Between the two sumatriptan
groups the respective changes at the same dose of sumatriptan were
evaluated with a Student's t-test. Statistical significance was accepted at
P < 0.05 (two-tailed).

Drugs

Apart from the anaesthetics, azaperone and metomidate (both from Janssen Pharmacæutica, Beerse, Belgium), the drugs used in this study
were: 5-HT creatinine sulphate (Sigma, St. Louis, Mo, USA), sumatrip-