# Potentiation of the cerebrovascular response to intra-arterial 5-hydroxytryptamine

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EIDELMAN, B. H., A. D. MENDELOW, T. A. MCCALDIN, AND D. S. BLOOM. *Potentiation of the cerebrovascular response to intra-arterial 5-hydroxytryptamine*. Am. J. Physiol. 234(3): H300–H304, 1978 or Am. J. Physiol.: Heart Circ. Physiol. 3(3): H300–H304, 1978. – Infusion of 5-hydroxytryptamine (5HT) into the internal carotid artery of normal baboons was not accompanied by alteration of gray matter cerebral blood flow. In animals pretreated with depot estrogen and progesterone (dosage equivalent to oral contraceptive preparations), infusion of 5HT produced a marked decrease in gray matter blood flow. A similar decrease in flow was obtained when the 5HT was infused with a concentrate of β-lipoprotein. Steroid substances appear to enhance the cerebrovascular constrictor responses to 5HT. A further series of six experiments has shown that the monoamine oxidase inhibitor tranylcypromine similarly produced constrictor responses to 5HT. It is possible that the steroids, the β-lipoprotein, and the tranylcypromine produced constrictor responses to 5HT by the same mechanism (inhibition of cerebrovascular monoamine oxidase).

*Papio ursinus;* cerebral blood flow; xenon-133; estrogen; serotonin; progesterone; β-lipoprotein; monoamine oxidase; tranylcypromine

IN SPITE OF extensive investigations no clear-cut understanding of cerebral vasospasm has emerged. Serotonin (5-hydroxytryptamine, 5HT) has been implicated in the genesis of the cerebral vasoconstriction accompanying the prodromal phase of migraine (1, 18). However, studies on the effect of intra-arterially administered 5HT on cerebral perfusion (as measured by isotope-clearance methods) reveal only moderate (35) or no change in perfusion (6, 26). Additional factors are probably operative in the migrainous patient.

It is well documented that migraine is more common in women taking contraceptives involving estrogens and progesterone (18). These steroid substances may be capable of potentiating the cerebral vasoconstrictor response to 5HT and may therefore precipitate the prodromal phase of migraine. In addition, migraine has been associated with hyperlipidemia (19). We have previously shown, in vitro, that an extract of β-lipoprotein potentiates the pressor response to norepinephrine (NE) (2). It is possible that a similar potentiation in vivo with 5HT could precipitate migraine.

The present experiments investigating the hypothesis that 5HT acts synergistically with steroids and β-lipoprotein in the cerebral circulation. The possibility that these substances may act by inhibition of cerebrovascular monoamine oxidase (MAO) has also been investigated.

## MATERIALS AND METHODS

Cerebral blood flow (CBF) was measured in 21 pentobarbital-anesthetized baboons by the intra-arterial xenon-133–injection method previously described (17). The carotid bifurcation was exposed and the lingual artery was cannulated for injection of xenon-133. All other branches of the external carotid artery were ligated. A highly collimated scintillation detector, 50 mm in diameter, was mounted over the parietal region to measure the uptake and clearance of the xenon-133. The skin and temporalis muscle were reflected from the area under the scintillation detector to exclude possible radiation from these sources. The xenon-133–clearance curve was analyzed into two monoexponential components representing blood flow through cerebral gray and white matter (13).

The bladder was catheterized and allowed to drain freely throughout the experiment. Rectal temperature was maintained between 35 and 38°C. Pulsatile and mean blood pressures (MBP), the analog xenon-133–clearance curve, endotracheal $CO_2$ percentage, and EEG were recorded on a Beckman Dynograph recorder.

The effects of intra-arterial 5HT infused at 2.5 and 5 μg/kg per min were studied in three groups of animals.

*Group 1*: controls (of both sexes)

*Group 2*: female animals pretreated 1 wk prior to the experiment with an intramuscular injection of depot estrogen (estradiol valerate, 2.5 mg; Schering) and progesterone (medroxyprogesterone acetate, 25 mg; Upjohn)

*Group 3*: normal animals (of both sexes) in which the 5HT was infused together with a concentrated β-lipoprotein extract of normal baboon plasma. This extract was obtained by ultracentrifugation methods previously described (2, 3).

A similar protocol was adopted for each experiment. After the surgery was complete the animals were allowed to stabilize for 30 min. Then, with a slow infusion of 0.1 ml/min of saline into the internal carotid, two base-line CBF measurements were made. The 5HT was then infused via the same route at 2.5 or 5.0 μg/kg per


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min. CBF was redetermined 10 min later. Base-line measurements (saline only) were repeated at intervals during the experiment after the 5HT had been discontinued for 30 min. Throughout these and subsequent experiments, arterial blood $Pco_2$, $Po_2$, pH, and mean pressure were maintained within normal limits (12, 20).

In a further study of six baboons the effect of 5HT on cerebral grey ($f_g$), white ($f_w$), and mean weighted average ($\bar{f}$) (14) blood flows were investigated during infusion of the MAO inhibitor tranylcypromine (SKF). The animals were initially sedated with phencyclidine (Sernylan; Bio-Ceutic), then intubated and ventilated. Anesthesia was maintained with thiopentone (Intraval-Sodium; Maybaker) by continuous intravenous infusion. The EEG was recorded throughout the experiment and was used to measure and control the level of anesthesia, which was maintained at a constant level of light sedation throughout. CBF was measured by the intracarotid xenon-133-clearance method.

After two initial base-line CBF values had been obtained, a 2 mM solution of the monoamine oxidase inhibitor tranylcypromine was infused into the internal carotid artery at a rate of 0.1 ml/min, and CBF was measured at least 10 min after commencing the infusion. On completion of this control CBF measurement, 5HT was infused at rates of 1.0 and 2.5 $\mu$g/kg per min, into the internal carotid artery, and cerebral blood flow was measured during the course of each infusion. Tranylcypromine infusion was maintained throughout.

## RESULTS

*1) Controls.* Intracarotid infusion of 5HT produced no significant alteration in cerebral gray matter blood flow ($f_g$). (See Table 1.)

*2) Estrogen and progesterone.* The mean base-line $f_g$ in the estrogen-progesterone animals was not significantly different from the value in the controls. With infusion of 5HT at 2.5 and 5.0 $\mu$g/kg per min, $f_g$ significantly decreased ($P < 0.025$ in both cases) (Table 1).

*3) $\beta$-Lipoprotein.* The mean base-line $f_g$ during $\beta$-lipoprotein infusion was higher than the value in the control group of animals. Administration of 5HT at 2.5 $\mu$g/kg per min produced a significant decrease in $f_g$ ($P < 0.025$). After the infusion there was a significant increase in the plasma $\beta$-lipoprotein level from 31.0 ± 5.1 to 56.6 ± 5.3 mg/100 ml in this group.

There was no significant alteration in arterial $Pco_2$, $Po_2$, pH, or mean blood pressure during the experiment in any of the groups of animals. Thus, these variables could not have contributed to the change in $f_g$ ($\Delta f_g$) found with 5HT. These $\Delta f_g$ values are shown in Fig. 1. The asterisk denotes that a significant difference was found in the $\Delta f_g$ values for the estrogen plus progesterone- and $\beta$-lipoprotein-treated animals when compared to the values for the same 5HT dose in the controls.

*4) Monoamine oxidase inhibition.* The resting values for $\bar{f}$, $f_g$, and $f_w$ were 31.2, 49.0, and 11.9 ml/min per 100 g of tissue, respectively. There was no significant change following infusion of tranylcypromine when the respective recorded values for $\bar{f}$, $f_g$, and $f_w$, were 31.5, 50.5, and 11.3 ml/min per 100 g (Fig. 2). However, infusion of 5HT at 1.0 and 2.5 $\mu$g/kg per min resulted in a significant decrease in $\bar{f}$, $f_g$, and $f_w$. The respective values and significance levels are shown in Table 2. There was no significant alteration in arterial $Pco_2$, $Po_2$, pH, or MBP during the experiment in any of the animals.

TABLE 1. *Gray matter blood flow*

| | Base Line | 5HT | |
|---|---|---|---|
| | | 2.5 | 5.0 $\mu$g/kg per min |
| Controls | 59.3 ± 9.5 | 59.0 ± 11.4 | 58.9 ± 14.4 |
| Est + progest pretreatment | 57.5 ± 3.6 | 43.9 ± 6.2*† | 45.7 ± 2.7*† |
| With $\beta$-lipoprotein infusion | 78.4 ± 11.4 | 43.3 ± 6.8*† | |

Values are means (±SE) of gray matter CBF ($f_g$) before and during infusion of 2.5 and 5.0 $\mu$g/kg per min of 5HT in control animals, estrogen- and progesterone-treated animals, and with infusion of $\beta$-lipoprotein. * $P < 0.025$ compared with control. † $P < 0.025$ compared with base line.

## DISCUSSION

The results of the present study indicate that intra-arterial administration of 5HT does not cause any significant change in gray matter perfusion. The effects of 5HT on the cerebral circulation have been extensively studied, but results are conflicting. Experiments in which 5HT has been applied topically to the adventitial surface either in vivo (15) or in vitro (34) have indicated that 5HT exerts a marked constrictor effect on cerebral blood vessels. Similarly, flowmeter evaluation of the effects of intra-arterial 5HT have indicated a cerebral vasoconstrictor action (10), although Swank and Hissen

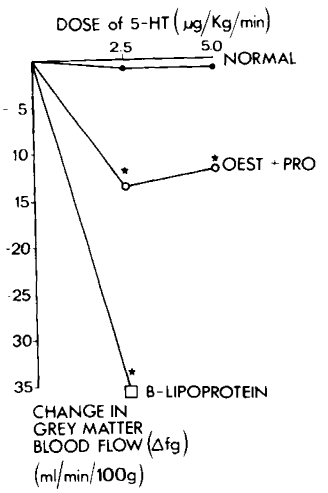


FIG. 1. Change in gray matter blood flow with infusion of 5HT. Mean changes of cerebral gray matter blood flow ($\Delta f_g$) with intracarotid infusion of 5HT are shown in normal animals (*closed circles*), in estrogen- and progesterone-treated animals (*open circles*), and with $\beta$-lipoprotein infusion (*open square*). $\Delta f_g$ values show no change in normals, but a decreased flow in treated animals. Asterisk denotes a significant difference between $\Delta f_g$ (normal) and $\Delta f_g$ (experimental) at all doses of 5HT ($P < 0.025$).

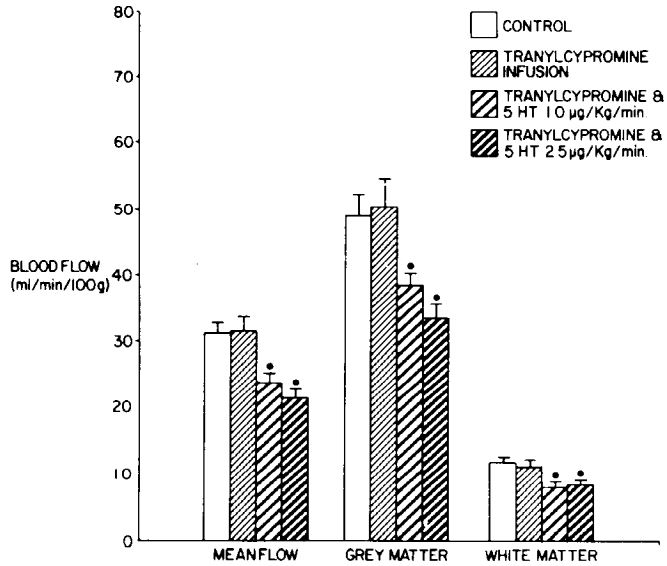


FIG. 2. Effect of 5HT infusion at 1.0 and 2.5 µg/kg per min on cerebral blood flow during infusion of MAO inhibitor tranylcypromine. All values are means ± SE. Symbol ● indicates $P < 0.05$ compared to control value using Student paired $t$-test.

TABLE 2. *Effect of 5HT on blood flow after infusion of tranylcypromine*

| | Grey Matter Flow | Mean Weighted Flow | White Matter Flow |
|---|---|---|---|
| | *milliliters per minute per 100 g* | | |
| Base line (control) | 49.0 ± 3.25 | 31.2 ± 1.65 | 11.9 ± 0.67 |
| Tranylcypromine alone | 50.5 ± 4.33 | 31.5 ± 2.41 | 11.3 ± 1.05 |
| Tranylcypromine + 5HT, 1.0 µg/kg per min | 38.7 ± 1.76*† | 23.9 ± 1.31*† | 8.0 ± 0.98*† |
| Tranylcypromine + 5HT, 2.5 µg/kg per min | 33.7 ± 2.20*† | 21.6 ± 1.35*† | 8.4 ± 0.73*† |

* $P < 0.05$ compared to control (paired $t$-test). † $P < 0.05$ compared to tranylcypromine also (paired $t$-test).

(33) reported dilatation. Isotope-clearance studies that measure cerebral perfusion have not shown any consistent response (6, 26). The minimal effect of 5HT on cerebral perfusion may relate to a differential effect of 5HT on intracerebral and extracerebral resistance vessels. Deskmukh and Harper (6) have shown that 5HT may produce constriction of extraparenchymal vessels without alteration of cerebral perfusion and have suggested that 5HT constricts only these vessels while compensatory autoregulatory changes in the intracerebral vessels maintain cerebral perfusion. Similar results, with use of a different technique, have been found by Rapela and Martin (27).

A further factor which may reduce the effect of intra-arterially administered 5HT relates to an endothelial barrier for this substance. The endothelial lining of cerebral vessels has been shown to contain monoamine oxidase (MAO) which is capable of 5HT degradation (11). It was therefore postulated that the smaller intracerebral vessels may not respond to arterial 5HT because of this barrier between blood and smooth muscle cells.

Furthermore, any 5HT that does diffuse across the barrier may be taken into the smooth muscle cells in a manner akin to the extraneuronal uptake of norepinephrine (NE) (4). It has been recently demonstrated that inhibition of the NE uptake process enhances the cerebral vasoconstrictor response to NE (16, 22). It is possible that similar mechanisms take up 5HT and reduce its constrictor action.

Thus, intra-arterially administered 5HT may be prevented from acting at the smaller intracerebral arterioles by either an endothelial blood-brain barrier or an avid extraneuronal uptake process.

*Steroids.* 5HT has been implicated in the genesis of migraine headache in which a marked decrease in cerebral blood flow has been shown in the prodromal phase (24, 25, 31). The lack of influence of intra-arterially infused 5HT suggests that other factors must be present to potentiate the 5HT effect. The results of the present study indicate that the two steroids, estrogen and progesterone, and the β-lipoprotein-cholesterol complex are capable of potentiating the constrictor actions of the 5HT. These results are in keeping with the clinical findings of Leviton and Camenga (19), who describe an increased incidence of migraine in association with pre-β-hyperlipidemia. In addition, migraine is known to be more common in women taking contraceptive pills (18). We have also demonstrated that corticosterone enhances cerebrovascular sensitivity to 5HT (23). Therefore, the steroid group of substances seems to potentiate the cerebrovascular constrictor effects of 5HT. In the present study, this constrictor effect was similar at all 5HT doses used. It may be that we have been working at the top end of the 5HT dose-response curve and that smaller doses would have produced a graded effect.

*Monoamine oxidase inhibition.* Pharmacological inhibition of cerebrovascular MAO activity resulted in decreased CBF with infusion of 5HT similar to that obtained with steroids. This suggests that all these compounds may have produced their effects by MAO inhibition, a hypothesis supported by the fact that cerebrovascular endothelium and cerebrovascular smooth muscle are rich in MAO (11, 16, 17). As inhibition of MAO at either site would allow a higher concentration of 5HT at receptors, any substance interfering with MAO activity would potentiate the vasoconstrictor action of 5HT. The pharmacological action of tranylcypromine in this respect is direct and in this study has produced 5HT constriction. Similarly, steroid hormones have been shown to inhibit MAO activity (21), and, more specifically, estradiol inhibits vascular smooth muscle uptake of 5HT (4). Thus, the potentiating action of steroids on 5HT may be explained by MAO inhibition.

In addition, steroid substances are also known to inhibit directly extraneuronal uptake of NE (28). This is analogous with the steroid effect on smooth muscle 5HT uptake (4). The steroids may thus decrease NE

uptake and, consequently, increase the concentration of NE in the tunica media. This increased NE concentration may then potentiate the effects of the infused 5HT (5). In addition, we have shown, in vitro, that an extract of β-lipoprotein potentiates the pressor response to NE (2).

This evidence suggests that steroids potentiate the cerebrovascular action of 5HT (and possibly other amines) by inhibition of the MAO degradative enzyme. The results of the present study support this hypothesis.

Recent evidence has shown that platelet MAO levels are decreased in migraine sufferers, particularly during attacks (8, 29). This is of particular relevance to the role of 5HT in the genesis of migraine, in which a fall in the 5HT of both plasma (32) and platelets (1) has been documented in the headache phase. CBF changes in migraine have been well demonstrated (25), with a decrease in flow occurring in the prodromal phase and an increase in the headache phase. These changes in CBF may be due to the alteration in the blood-brain barrier permitting circulating 5HT to produce constriction of intraparenchymal arteries. The fall in circulating 5HT reported by Somerville (32) may be due to migration of 5HT from the circulation through these defective barriers. According to this hypothesis, it may not be the circulating amine concentration that primarily influences cerebral blood flow. Rather, it is the state of the enzymatic blood-brain barrier and the cerebrovascular smooth muscle that determines the vascular response to 5HT. A similar change in the cerebrovascular sensitivity to intravascular norepinephrine after inhibition of catechol-*O*-methyltransferase has been reported previously (22).

The present study shows that estrogen and progesterone, β-lipoprotein, and MAO inhibition all produce a decreased CBF with intracarotid 5HT infusion. Changes in the metabolism of amines in cerebral vessels fit in well with the finding that migraine sufferers have low MAO activity (8, 29). Furthermore, they provide an explanation for the effect of steroid hormones in migraine (9) and are in agreement with the observation that 5HT plays a role in the pathogenesis of this disorder (30). One of the important factors in migraine may therefore be a change in 5HT metabolism in the cerebral vessel wall itself.

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