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Effects of Serotonin and *L*-5-Hydroxytryptophan on Plasma Renin Activity in Rats[1]

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BARNEY, C. C., R. M. THREATTE, D. C. KIKTA AND M. J. FREGLY. *Effects of serotonin and l-5-hydroxytryptophan on plasma renin activity in rats.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 895–900, 1981.—The effects of dipsogenic doses of *l*-5-hydroxytryptophan (5-HTP) and serotonin on plasma renin activity (PRA), blood pressure, and body temperature were determined in unanesthetized female rats. Both serotonin (2 mg/kg, s.c.) and 5-HTP (25 mg/kg, s.c.) induced six-fold increases in PRA measured 1 hr after drug administration. The central and peripheral decarboxylase inhibitor, benserazide (30 mg/kg, s.c.), as well as the peripheral decarboxylase inhibitor, carbidopa (6.5 mg/kg, s.c.), prevented the increase in PRA associated with administration of 5-HTP. This suggests that 5-HTP must be converted to serotonin peripherally to increase PRA. At the doses used, serotonin decreased mean blood pressure and colonic temperature of unanesthetized rats while 5-HTP was without effect. The increase in PRA induced by 5-HTP does not appear, therefore, to be a response to either hypotension or a decrease in colonic temperature. Since 5-HTP must be converted to serotonin to initiate both a drinking response and an increase in PRA, the results suggest that the decrease in blood pressure and colonic temperature following administration of serotonin may not be important in induction of the drinking response and the increase in PRA. The mechanism by which activation of the renin-angiotensin system occurs following peripheral administration of either 5-HTP or serotonin remains for further study.

Heart rate Blood pressure Decarboxylase inhibitors Carbidopa Benserazide Colonic temperature Tail skin temperature

PREVIOUS studies from this laboratory have shown that *l*-5-hydroxytryptophan (5-HTP) is dipsogenic when administered peripherally to rats [7,19]. On the other hand, d-tryptophan, *l*-tryptophan, acetyltryptophan, and melatonin were all without significant effect on water intake of rats [7]. Serotonin, which is formed from 5-HTP by 5-hydroxytryptophan decarboxylase [14], is also dipsogenic in rats [10, 12, 15]. Serotonin-induced drinking is associated with an increase in plasma renin activity (PRA) [15] which eventually leads to formation of increased amounts of angiotensin II, a potent dipsogenic hormone [5,6]. Previous results from this laboratory have also indicated that 5-HTP may likewise induce drinking via activation of the renin-angiotensin system since the dipsogenic effect of 5-HTP can be attenuated by the β-adrenergic antagonist, propranolol, and by the angiotensin I converting enzyme inhibitor, captopril [19]. It also appears likely that 5-HTP must be converted to serotonin before induction of drinking can occur in the rat [10].

The present study tested the effect of serotonin, 5-HTP, and 5-HTP plus decarboxylase inhibitors on PRA of rats. The results support the hypothesis that 5-HTP induces drinking in rats via conversion to serotonin and subsequent activation of the renin-angiotensin system. In addition, the cardiovascular effects of doses of serotonin and 5-HTP that increased PRA were investigated to determine if hypotension may be a causative factor. Finally, the effects of serotonin and 5-HTP on body temperature were measured to determine if the increase in PRA and water intake caused by these agents could be correlated with any thermal effects.

METHOD

Female rats of the Blue Spruce Farms (Hooded) strain weighing initially from 200–250 g were used unless otherwise indicated. The rats were housed 4 to a cage (except following arterial cannulations when they were housed individually) in a room maintained at 24±1°C and illuminated

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from 7 a.m. to 7 p.m. Rats were provided with Purina Laboratory Chow and tap water ad lib.

Experiment 1. Effect of Serotonin on Plasma Renin Activity

Twelve rats were used for this experiment. Six rats were injected with 2 mg serotonin (5-Hydroxytryptamine-Creatinine Sulfate, Sigma Chemical Company)/kg, s.c. and the remaining 6 rats were injected with 1 ml saline/kg, the vehicle used to solubilize the serotonin. One hour later, during which time the rats had access to water but not food, each rat was anesthetized with methoxyflurane (Penthrane®, Abbott Laboratories). A 3.0 ml blood sample was obtained by cardiac puncture and collected in cooled centrifuge tubes containing soldium-EDTA at a final concentration of 1.0 mg/ml blood. The blood was cooled in an ice bath immediately after collection and centrifuged at 12,000×G for 30 min at 4°C. Plasma was removed and stored at −20°C until determination of PRA in duplicate by the modified method of Pettinger *et al.* [18] using an angiotensin I (^{125}I) radioimmunoassay kit (Rianen®, New England Nuclear Corp.). The data were analyzed statistically by means of a *t*-test with significance set at the 95% confidence level [4].

Experiment 2. Effect of 5-Hydroxytryptophan, Alone and with Decarboxylase Inhibitors, on Plasma Renin Activity

Forty-one rats were used for this experiment. Each rat received two injections. Six rats (controls) received two injections of saline (1 ml/kg, s.c.). Seven rats received saline (1 ml/kg, s.c.) followed by 25 mg 5-HTP (5-Hydroxytryptophan Monohydrochloride, Calbiochem Behring Corp.)/kg, s.c. Seven rats received 6.5 mg carbidopa (gift of Dr. C. A. Stone, Merck, Sharpe, and Dohme Research Laboratories)/kg, s.c. followed by 25 mg 5-HTP/kg, s.c. Seven rats received 30 mg benserazide (gift of Dr. C. Kadzielawa, Department of Pharmacology and Therapeutics, University of Florida, College of Medicine)/kg, s.c. followed by 25 mg 5-HTP/kg, s.c. One hour later, during which time the rats had access to water but not food, each rat was anesthetized with methoxyflurane, a blood sample collected, and PRA determined as in experiment 1. Data were analyzed statistically by an analysis of variance [4] with significance set at the 95% confidence level. Comparisons between individual means were made by the *t*-test using the pooled variance from the analysis of variance [4].

Experiment 3. Effect of 5-HTP and Serotonin on Blood Pressure and Heart Rate

Twenty-two rats were used for this experiment. The left common carotid artery of each rat was cannulated as follows. The rats were anesthetized with 39 mg pentobarbital (Pentosol®, W.A. Bulter Co.)/kg, i.p. and also administered 0.04 mg atropine sulfate (Eli Lilly and Co.)/rat, s.c. A length of PE-50 tubing with a small bubble 2.8 cm from the end was inserted into the artery until it reached the aortic arch (confirmed at autopsy) and sutured in place. The tubing was exteriorized at the back of the neck and sealed with a stainless steel stylette. The tubing was filled with heparinized (100 units/ml) saline. Upon completion of the surgery each rat was injected with 300,000 units of penicillin (Bicillin L-A®, Wyeth Laboratories, Inc.). The cannulae were kept in 70% ethanol and the surgical instruments in 0.023% benzalkonium chloride prior to use. Following surgery the cannulae were flushed with heparinized saline every 3 days. Rats were allowed to recover at least 6–7 days prior to use.

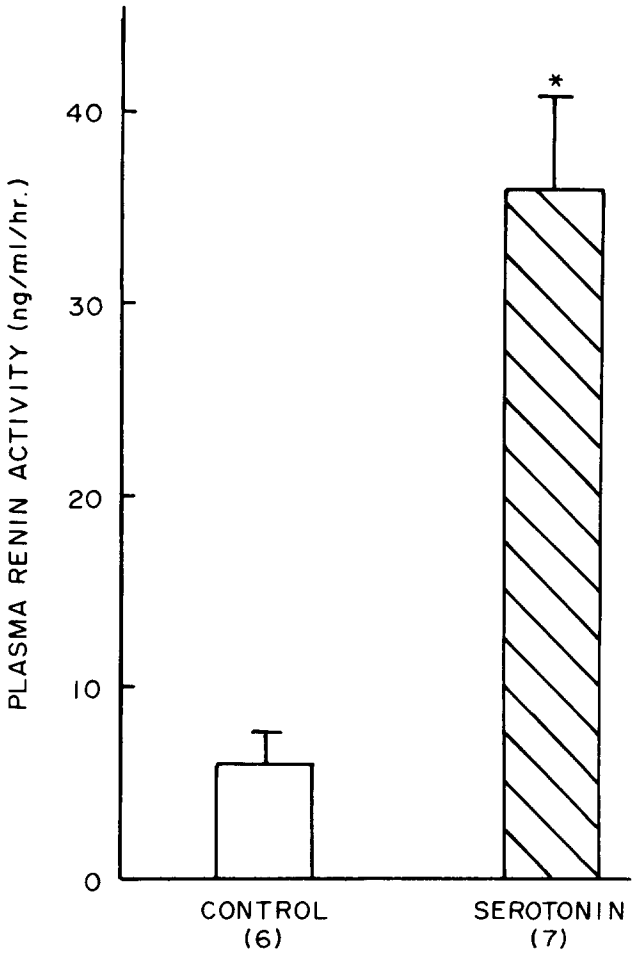

FIG. 1. Mean plasma renin activity of control and serotonin-treated (2 mg/kg, s.c.) rats 1 hr after treatment. One standard error is set off at each mean. Number of rats is shown in the parentheses. * indicates significant difference ($p<0.01$) from control group.

For measurements of blood pressure and heart rate, the cannula of each rat was filled with heparinized saline and connected via PE-50 tubing to a pressure transducer (Statham P23dB). Blood pressures of conscious, unrestrained rats, having access to water but not food, were measured by means of a Grass polygraph. Blood pressure and heart rate were measured in 6 saline-treated, control rats; 8 serotonin-treated rats (2 mg/kg s.c.) and 8 5-HTP-treated rats (25 mg/kg, s.c.). After the cannulae were connected to the transducers, the rats were allowed 30 min to adjust. A control (0 time) measurement of blood pressure was made following which the rats were injected. Following administration of either saline or drug, blood pressures were recorded at 5 min intervals for the first 30 min and at 15 min intervals for the next 90 min. The mean aortic blood pressures and heart rates were determined from the pressure tracings. The data were analyzed statistically as in experiment 2.

Experiment 4. Effect of 5-HTP and Serotonin on Colonic and Tail Skin Temperatures

In the first study 10 female rats of the Blue Spruce Farms (Sprague Dawley) Strain weighing 270 to 310 g were used.

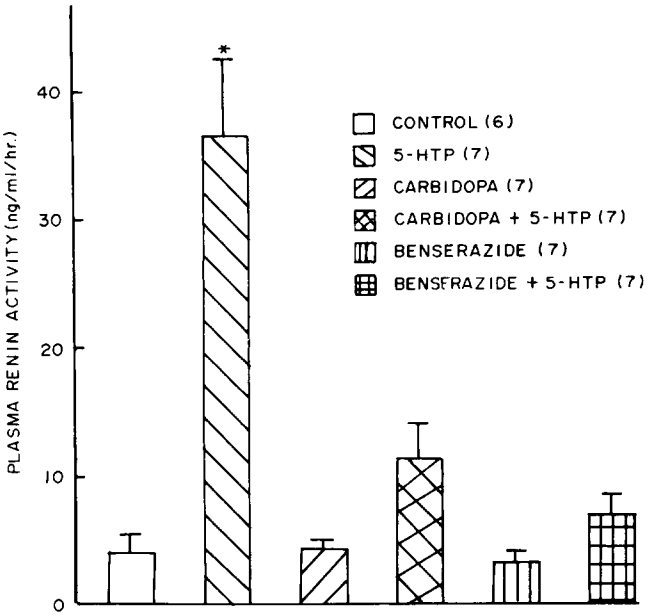

FIG. 2. Mean plasma renin activity of control rats and rats treated with 5-HTP (25 mg/kg, s.c.), carbidopa (6.5 mg/kg, s.c.), and benserazide (30 mg/kg, s.c.) alone or in combination. Plasma renin activity was measured 1 hr after treatment. One standard error is set off at each mean. Number of rats is shown in the parentheses. * indicates significant difference ($p<0.01$) from control group.

Tail skin and colonic temperatures were measured at an ambient temperature of 25±1°C in rats restrained in tunnel-type cages with wire mesh covers [1]. Colonic temperature was measured with a copper-constantan thermocouple inserted 6 cm beyond the anus and taped to the tail. Tail skin temperature was measured with a copper-constantan thermocouple woven into a gauze sponge and taped to the tail so that the thermocouple contacted the skin near the base of the tail. Temperatures were recorded by a potentiometer every 6 min.

The rats were allowed 1 hr to adjust. Control measurements were then made for 30 min. At the end of the control period, 5 rats (controls) were administered 1 ml saline/kg, s.c. and 5 rats were administered 25 mg 5-HTP/kg, s.c. Measurements were then continued for 114 min. Data were analyzed statistically as in experiment 1.

In the second study 12 female rats of the Blue Spruce Farms (Sprague Dawley) Strain weighing 260 to 320 g were used. The first study was repeated except that 6 rats received 1 ml saline/kg s.c. and 6 rats received 2 mg serotonin/kg s.c.

RESULTS

Experiment 1

Plasma renin activity was significantly elevated ($p<0.01$) above that of control rats 1 hr after administration of 2 mg serotonin/kg (Fig. 1). The serotonin-treated rats had a mean PRA which was approximately 6 times that of the control rats.

Experiment 2

Plasma renin activities of rats administered 5-HTP alone and in combination with the decarboxylase inhibitors, carbidopa and benserazide, are shown in Fig. 2. One way analysis of variance indicated a significant effect of treatment ($p<0.01$). The group treated with 5-HTP alone exhibited a significant ($p<0.01$) increase in PRA compared to the control group. Neither carbidopa nor benserazide, when administered alone, had a significant effect on PRA. However, both carbidopa and benserazide prevented the increase in PRA characteristically accompanying administration of 5-HTP.

Experiment 3

The changes in heart rate and mean blood pressure of awake, unrestrained rats after administration of saline, 5-HTP, or serotonin are shown in Fig. 3A and B respectively. Prior to administration of either saline or drug solutions, there was no significant difference in either heart rate (control=424±12 (S.E.) beats/min, 5-HTP=417±16 beats/min, serotonin=419±8 beats/min) or mean blood pressure (control=126±5 mm Hg, 5-HTP=122±6 mm Hg, serotonin=130±4 mm Hg) among the three groups. Throughout the measurement period both the control and 5-HTP-treated groups showed little change in mean blood pressure with no significant difference between the 5HTP-treated and control groups at any point (Fig. 3B). Administration of serotonin, however, was accompanied by a significant ($p<0.05$ to $p<0.01$) and long-lasting fall in mean blood pressure (Fig. 3B). Although the heart rate of the serotonin-treated group appeared more variable than the other two groups, there was no significant effect of either 5-HTP or serotonin on heart rate (Fig. 3A).

Experiment 4

Administration of 5-HTP to rats had no significant effect on either colonic (Fig. 4A) or tail skin (Fig. 4B) temperatures. In contrast, administration of serotonin led to a significant ($p<0.05$) decrease in colonic temperature (Fig. 5A). The rats treated with serotonin had significantly lower colonic temperatures as compared to the control group from 30 to 105 min after administration. Although colonic temperature was lower in the serotonin-treated group, there was no significant change in tail skin temperature (Fig. 5B).

DISCUSSION

Both serotonin and its precursor, 5-hydroxytryptophan [11], increased water intake when administered peripherally to rats [7, 10, 12, 15, 19]. One possible mechanism for the dipsogenic effect of both 5-HTP and serotonin is activation of the renin-angiotensin system [5, 6, 15, 19]. The results reported here lend support to this possibility. Administration of serotonin to rats led to a large increase in plasma renin activity (Fig. 1) in confirmation of the report of Meyer *et al.* [15]. Plasma renin activity was also increased by administration of 5-HTP (Fig. 2). Administration of either the central and peripheral decarboxylase inhibitor, benserazide [2], or the peripheral decarboxylase inhibitor, carbidopa [20], acted to prevent the increase in PRA accompanying administration of 5-HTP. Thus, it appears that 5-HTP must first be converted peripherally to serotonin before an effect on plasma renin activity is observed.

It is of interest to compare the effects of serotonin and 5-HTP on PRA with their dipsogenic effects. In the present study administration of either 2 mg serotonin/kg or 25 mg 5-HTP/kg resulted in equal increases in PRA measured 1 hr after drug administration. These doses also increased water

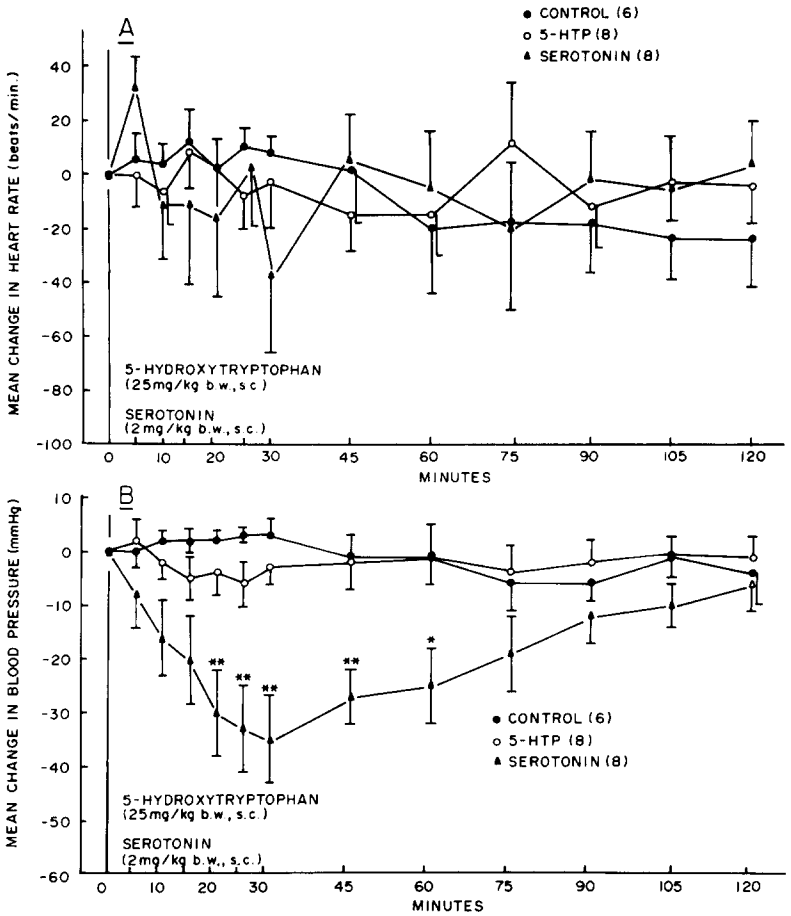

FIG. 3. Mean change in heart rate (A) and mean change in mean blood pressure (B) of control, 5-HTP-treated (25 mg/kg, s.c.) and serotonin-treated (2 mg/kg, s.c.) rats. One standard error is set off at each mean. Number of rats is shown in the parentheses. ** and * indicate significant differences from control group ($p<0.01$ and $p<0.05$, respectively).

intake measured during 2 hr after their administration [7,10]. In addition, the decarboxylase inhibitors, benserazide and carbidopa, prevented both the increase in water intake [10] and the increase in plasma renin activity induced by administration of 5-HTP. Benserazide and carbidopa by themselves had no significant effect on either PRA (Fig. 2) or water intake [10]. These findings, together with the observation that propranolol and captopril attenuate the dipsogenic effect of 5-HTP [19], are consistent with the concept that 5-HTP induces drinking in rats by its conversion to serotonin peripherally and subsequent activation of the renin-angiotensin system.

The mechanism by which 5-HTP increases plasma renin activity in the rat appears to be different from that in the dog. In the latter, 5-HTP, at a dose of 20 mg/kg, induced an increase in PRA which was prevented by administration of benserazide but not carbidopa [8,22]. In fact, carbidopa plus 5-HTP gave a greater increase in PRA than did 5-HTP alone [8,22]. As in the present study, no effect of either carbidopa or benserazide on PRA was observed [22]. These data suggest that, in the dog, 5-HTP increases PRA by its conversion centrally to serotonin. Thus, a species difference may exist between the rat and the dog with respect to the area of the body at which decarboxylation of 5-HTP to serotonin initiates an increase in PRA. Further experiments on the mechanism by which 5-HTP increases PRA in a variety of species would be of interest in this regard.

One mechanism by which PRA can be increased, leading to an increase in water intake, is in response to acute hypotension [15]. Alternatively, it has been suggested that the drinking response accompanying administration of hypotensive agents is due to the hypotension itself and not the resulting increase in PRA [9]. Blood pressures of conscious, unrestrained rats were measured following administration of either serotonin or 5-HTP to determine whether the PRA and drinking responses to these agents were correlated with a fall in blood pressure. As shown in Fig. 3, administration of serotonin, but not 5-HTP, was accompanied by a significant fall in mean blood pressure. These two different responses of blood pressure occurred at doses of serotonin and 5-HTP which induced similar increases in PRA and water intake [7,10]. Thus, serotonin does have a hypotensive effect, as has been reported previously [3]. This does not appear to be the case for 5-HTP. The difference in the re-

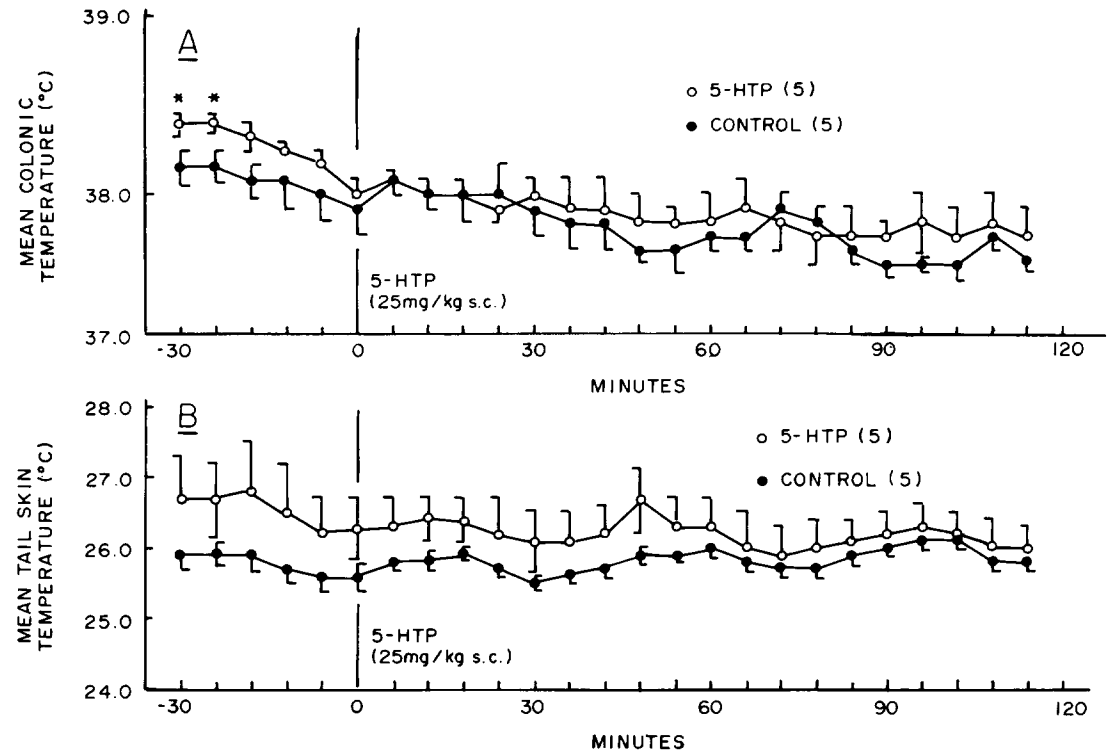

FIG. 4. Mean colonic (A) and tail skin temperatures (B) of control and 5-HTP-treated (25 mg/kg, s.c.) rats. One standard error is set off at each mean. Number of rats shown in parentheses. * indicates significant difference ($p<0.05$) from control group.

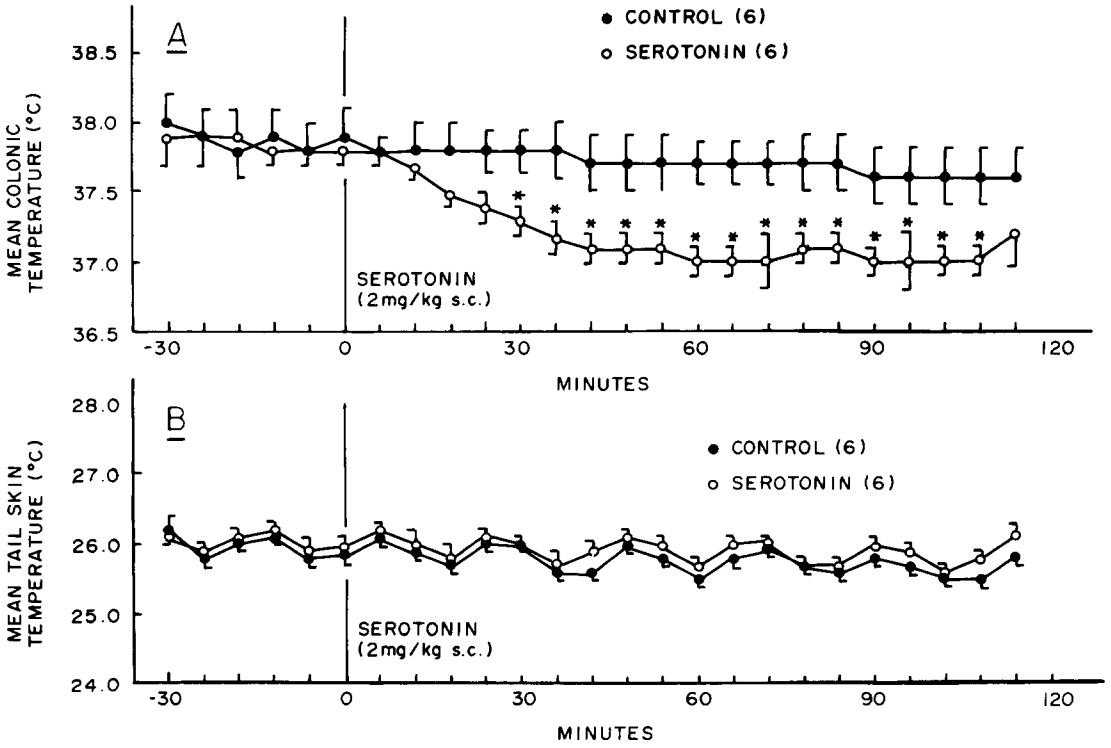

FIG. 5. Mean colonic (A) and tail skin temperatures (B) of control and serotonin-treated (2 mg/kg, s.c.) rats. One standard error is set off at each mean. Number of rats is shown in parentheses. * indicates significant difference ($p<0.05$) from control group.

sponse of blood pressure to serotonin and 5-HTP is of interest since 5-HTP must be converted to serotonin before it affects either PRA or water intake. This suggests that the decrease in blood pressure may not be important in induction of both the drinking response and the increase in PRA accompanying administration of serotonin. The slow formation of serotonin by the body following administration of 5-HTP versus the rapid increase in serotonin levels of blood following peripheral administration of serotonin may account for the different effects on blood pressure. This may also explain the different time courses of the increase in water intake observed after administration of serotonin as compared with 5-HTP [7,10]. In addition, serotonin formed from administered 5-HTP can influence catecholaminergic neuronal function. Following uptake and decarboxylation of 5-HTP by catecholaminergic neurons, it may then displace endogenous catecholamines from storage granules [21]. Thus, a concomitant release of catecholamines may be responsible for the lack of hypotension following the administration of 5-HTP.

Both 5-HTP and serotonin are known to have effects on the thermoregulatory system [13,16]. It was of interest to determine if the doses of 5-HTP and serotonin which led to similar increases in PRA would alter body temperature in a similar manner. As with blood pressure, serotonin acted to decrease colonic temperature while 5-HTP was without effect (Figs. 4 and 5). The fall in colonic temperature following peripheral administration of serotonin is in agreement with previous findings [16]. The lack of an increase in tail skin temperature after injection of serotonin indicates that the fall in colonic temperature was probably related to a decrease in heat production rather than an increase in heat loss. It has been reported previously that serotonin acts to decrease oxygen consumption when administered peripherally (reviewed in [16]). Unlike serotonin, 5-HTP did not alter colonic temperature significantly. Although 5-HTP has been reported to induce hypothermia in rats, it was shown that the hypothermia occurred only at temperatures below the thermoneutral zone [13]. Thus, the increase in PRA induced by serotonin and its precursor 5-HTP, does not appear to be dependent on either the themoregulatory or blood pressure effects of these agents. The exact mechanism for the increase in PRA induced by serotonin and 5-HTP acting through serotonin, remains to be elucidated by further study.

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