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INHIBITION OF FIRING OF RAPHE NEURONES BY TRYPTOPHAN AND 5-HYDROXYTRYPTOPHAN: BLOCKADE BY INHIBITING SEROTONIN SYNTHESIS WITH Ro-4-4602

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Summary—In these studies, serotonergic neuronal activity was monitored by extracellular recording from identified cells in the midbrain dorsal raphe nucleus. The systemic administration of L-tryptophan, the initial precursor in the biosynthesis of brain serotonin (5-HT), was found to depress the firing of serotonergic neurones. The systemic administration of L-5-hydroxytryptophan, the immediate precursor of 5-HT, in combination with a low dose of Ro4-4602 (50 mg/kg, which inhibits mainly peripheral decarboxylase) was also found to depress raphe firing. The tryptophan-induced depression of raphe activity was prevented by pretreatment of the animal with an L-aromatic amino acid decarboxylase inhibitor, Ro4-4602, in a dose (800 mg/kg) sufficient to block the formation of 5-HT in raphe neurones. *p*-Chlorophenylalanine, another 5-HT synthesis inhibitor, was found to be ineffective in preventing the depression of raphe firing by tryptophan. However, previous studies have shown that *p*-chlorophenylalanine does not prevent the accumulation of 5-HT within the raphe perikarya following loading doses of tryptophan (AGHAJANIAN, KUHAR and ROTH, 1973). Raphe neurones were also inhibited by the local (microiontophoretic) administration of L-tryptophan or L-5-hydroxytryptophan. We conclude that (1) both 5-HT precursors (tryptophan and 5-hydroxytryptophan) inhibit raphe firing through their enzymatic conversion to 5-HT; (2) a local increase in 5-HT within the raphe perikarya is sufficient to lead to a decrease in raphe cellular activity.

Histochemical studies (DAHLSTRÖM and FUXE, 1965; FUXE, 1965; ANDÉN, DAHLSTRÖM, FUXE, LARSSON, OLSON and UNGERSTEDT, 1966; BJÖRKLUND, FALCK and STENEVI, 1971; UNGERSTEDT, 1971; KUHAR, AGHAJANIAN and ROTH, 1972a; AGHAJANIAN *et al.*, 1973) and combined lesion and biochemical studies (HELLER and MOORE, 1965; JOUVET, 1967; KOSTOWSKI, GIACALONE, GARATTINI and VALZELLI, 1968; ROSECRANS and SHEARD, 1969; KUHAR, ROTH and AGHAJANIAN, 1971, 1972b) have shown that there is a selective localization of serotonin (5-hydroxytryptamine; 5-HT) within a specific neuronal system whose cell bodies are located in the brainstem raphe nuclei. There is evidence that brain tryptophan hydroxylase, the rate limiting enzyme in the conversion of the initial precursor L-tryptophan to 5-HT (JEQUIER, LOVENBERG and SJOERDSMA, 1967), is located almost exclusively within the raphe system (KUHAR *et al.*, 1971). Brain 5-HT levels are increased by the administration of loading doses of tryptophan (HESS and DOEPFNER, 1961; ASHCROFT, ECCLESTON and CRAWFORD, 1965; WEBER and HORITA, 1965; FERNSTROM and WURTMAN, 1971); the greatest increase in 5-hydroxyindoles occurs in the midbrain area which contains the raphe nuclei (MOIR and ECCLESTON, 1968; KNOTT and CURZON, 1974). Loading doses of tryptophan also cause a selective enhancement in the fluorescence of serotonergic neurones (AGHAJANIAN and ASHER, 1971), the largest clusters of which are located in the midbrain dorsal raphe nucleus.

Recently, it has been demonstrated that tryptophan can be used as a selective histochemical marker for serotonergic neurones in single unit recording studies, based on the fact that only serotonergic neurones (e.g. in the dorsal raphe nucleus) can convert microiontophoretically applied tryptophan to 5-HT *in situ* (AGHAJANIAN and HAIGLER, 1974). Such identified serotonergic cells are characterized by a slow firing rate (approximately 1 spike/sec) and a regular rhythm. In addition, certain pharmacological characteristics of raphe neurones have been reported which include: (1) inhibition by lysergic acid diethylamide (LSD) applied both systemically and by microiontophoresis (AGHAJANIAN, FOOTE and SHEARD, 1968; AGHAJANIAN, HAIGLER and BLOOM, 1972; BRAMWELL and GONYE, 1973; MCGINTY, HARPER and FAIRBANKS, 1973; HAIGLER and AGHAJANIAN, 1974; MOSKO and JACOBS, 1974); (2) depression of firing by drugs or precursors which enhance the availability or concentration of 5-HT (AGHAJANIAN, GRAHAM and SHEARD, 1970; AGHAJANIAN, 1972; BRAMWELL, 1972; SHEARD, ZOLOVICK and AGHAJANIAN, 1972; BRAMWELL, 1974); and (3) depression of firing by 5-HT applied directly by microiontophoresis (AGHAJANIAN *et al.*, 1972; BRAMWELL and GONYE, 1973; HAIGLER and AGHAJANIAN, 1974). In other studies, cells in the median and pontine raphe areas have been described which have very different properties such as high rates of discharge and a mixture of excitatory as well as inhibitory responses to microiontophoretically applied 5-HT (COUCH,

1970). However, these cells cannot be assumed to be serotonergic in the absence of direct histochemical verification.

Based on observations that both 5-HT and compounds that increase the concentration or availability of brain 5-HT decrease the activity of serotonergic neurones, it has been proposed that there is an inverse relationship between 5-HT concentration and neuronal activity within the raphe (AGHAJANIAN *et al.*, 1970; AGHAJANIAN, 1972; AGHAJANIAN *et al.*, 1972; BRAMWELL, 1972; SHEARD *et al.*, 1972; BRAMWELL and GONYE, 1973). The depression in the firing of neurones in the dorsal raphe nucleus induced by several compounds which have been reported to increase the availability of 5-HT (e.g. tertiary tricyclic antidepressants and monoamine oxidase inhibitors) can be blocked by pretreatment with *p*-chlorophenylalanine (*p*-CPA) a 5-HT synthesis inhibitor (AGHAJANIAN *et al.*, 1970; SHEARD *et al.*, 1972). Thus, the action of both tricyclic antidepressants and monoamine oxidase inhibitors appears to be secondary to an accumulation of 5-HT. However, evidence that 5-HT precursors (i.e. tryptophan and 5-hydroxytryptophan; 5-HTP) inhibit raphe firing through their biochemical conversion to 5-HT is less clear. After the administration of tryptophan, there is a depression in the rate of firing of raphe neurones under the same conditions in which 5-HT concentration and fluorescence of raphe cells is increased (AGHAJANIAN, 1972). However, *p*-CPA was found to be ineffective in preventing the depression of raphe unit firing produced by tryptophan (AGHAJANIAN, 1972). Furthermore, treatment with *p*-CPA did not block the tryptophan-induced enhancement of raphe cell fluorescence (AGHAJANIAN and ASHER, 1971). Additional studies have indicated that the perikarya of raphe neurones are relatively resistant to the action of *p*-CPA, permitting continued conversion of 5-HT in this region after loading doses of tryptophan (AGHAJANIAN *et al.*, 1973). Thus, the question remains open as to whether precursors of 5-HT can induce a decrease in the firing rate of raphe neurones as a result of increased 5-HT accumulation within the perikarya of 5-HT neurones.

Previous work has suggested that following Ro4-4602, an aromatic amino acid decarboxylase inhibitor, an accumulation of 5-HTP in brain occurs within the perikarya and projection systems of serotonergic neurones (BÉDARD, CARLSSON, FUXE and LINDQVIST, 1971). Since the conversion of 5-HTP to 5-HT is prevented by the inhibition of decarboxylase, one may test whether or not the depression of raphe activity induced by tryptophan is ultimately due to the conversion of this amino acid to 5-HT. In order to test this hypothesis, the effect of Ro4-4602 on the tryptophan-induced depression in raphe cell firing was examined. Tryptophan was given by both systemic and microiontophoretic routes. The effects of 5-HTP were also investigated. In addition, levels of 5-HT and tryptophan in the midbrain raphe area and in the forebrain were measured under conditions similar to those in the electrophysiological experiments.

METHODS

Experimental subjects and single unit recording

Male albino rats (Charles River, 220–280 g) were used in these experiments. For purposes of recording, animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. Animals were kept anaesthetized with additional injections of chloral hydrate as needed throughout the experiment. In experiments where drugs were administered only by the systemic route, single barrel micropipettes (tip diameters: 1–2 μm) were used. Micropipettes were filled with 2 M NaCl saturated with fast green dye. The electrodes typically had an impedance of 4–6 MΩ when measured at 60 Hz. A burr hole was made in the midline of the skull over the midbrain raphe area (approximately 0·5–1·0 mm anterior to lambda). Electrodes were lowered into the midbrain raphe region with a hydraulic microdrive system. Electrode signals were passed through a high input-impedance amplifier and monitored on an oscilloscope. With optimal electrode placement a zone of electrical silence, which corresponded to the depth of the cerebral aqueduct, was encountered just dorsal to the dorsal raphe area. Integrated firing rate was recorded by means of a rate meter as previously described (AGHAJANIAN *et al.*, 1970). Body temperature was measured by a rectal probe, and was maintained between 36–37°C. Under these conditions, midbrain raphe units displayed a regular rhythm and rate of 0·5–2·5 spikes/sec, which is typical for histochemically identified 5-HT units (AGHAJANIAN and HAIGLER, 1974). Recording sites were marked by passing a 16 μA cathodal current through the recording barrel for 10 min which resulted in the deposition of a discrete spot of fast green dye (THOMAS and WILSON, 1965). The precise location of the electrode tip was confirmed by histological examination and compared with the previously established location of 5-HT-containing neurones in the dorsal raphe nucleus.

Microiontophoretic studies

For some of the microiontophoretic studies six barrel micropipettes were constructed using techniques similar to those described by CURTIS (1968), OLIVER (1971) and HAIGLER and AGHAJANIAN (1973). A single barrel micropipette, bent at a 20–30° angle, was joined to a five barrel micropipette (tip diameter 5–10 μm) with dental acrylic so that the tip of the single barrel (diameter 1–2 μm) projected 30–50 μm beyond the 5 barrel micropipette. This permitted relatively large amounts of tryptophan (100–200 nA) to be ejected from the multibarrel pipette over long periods of time (5 min or longer) without affecting the recording characteristics of the attached single barrel. The attached recording barrel was filled with a 2 M NaCl solution saturated with fast green. Drug solutions

were injected directly into various barrels of the 5 barrel electrode according to the method of TASAKI, TSUKAHARA, ITO, WAYNER and YU (1968). Two barrels of the five barrel micropipette were filled with 0·1 M tryptophan, pH 4·0 (Calbiochem) while another was filled with 0·05 M serotonin creatinine sulphate, pH 4·0 (Sigma Chemical Co.). A fourth barrel, filled with 4 M NaCl, was used as the balance channel. The central barrel was not filled. Impedances, measured at 60 Hz were 4–6 MΩ in the recording barrel, 10–12 MΩ in the balance channel and 60–150 MΩ in the drug barrels.

Circuitry for the iontophoretic ejection of drugs and current balance was as described by SALMOIRAGHI and WEIGHT (1967), except that a solid state system was used. Drugs were ejected from micropipette barrels by turning the direct current source from −12 nA (5-HT) or −60 nA (tryptophan) of retaining current to +10 nA (5-HT) or +100 nA (tryptophan) of ejecting current. For microiontophoretic studies with 5-HTP, five barrel micropipettes were used as previously described (AGHAJANIAN *et al.*, 1972). In these experiments, one of the side barrels was filled with 0·1 M 5-HTP (Sigma Chemical Co.) in distilled H_2O, titrated to pH 4·0 with 1 M tartaric acid. 5-Hydroxytryptophan was iontophoresed by turning the direct current source from −12 nA of retaining current to +20 or +30 nA of ejecting current.

The transport number, an index of the efficiency of iontophoretic drug ejection, was determined as previously described (HAIGLER and AGHAJANIAN, 1974) for tryptophan. While determining this value, it was observed that the ejection of tryptophan was proportional to current strength only when cationic currents and solutions of relatively low pH (3·5–4·0) were used. This is in agreement with the findings of CURTIS (1964), who suggested that substances of low solubility and/or low ionization behave as if positively charged; in such cases electro-osmosis tends to favour the ejection of cations. Nevertheless, at a pH of 4·0, the mean transport number for tryptophan was very low (0·0021 ± 0·0001 S.D., n = 7), resulting in the total ejection of approximately 1·3 pmol of tryptophan in 10 min. Using similar conditions, HAIGLER and AGHAJANIAN (1974) have reported the transport number for 5-HT to be 0·219. This suggests that iontophoretic and/or electro-osmotic release was 100 times more efficient for 5-HT than for tryptophan.

After microiontophoretically applying tryptophan and 5-HT to raphe cells Ro4-4602 (800 mg/kg, i.p.) was administered. Twenty to 60 min later, responses of raphe cells to the same ejection currents of tryptophan and 5-HT as in the pre-Ro4-4602 treated animals were observed. Recording sites were marked by ejecting fast green dye through the recording barrel as described above.

Biochemical studies

The effects of the drugs used in the above electrophysiological studies on the endogenous concentration of 5-HT or tryptophan in brain were also determined.

To measure the effects of drugs on 5-HT levels in brain, drugs were administered to rats as follows: (1) no drug (saline); (2) tryptophan (100 mg/kg, i.p.) 20 min or 1 hr prior to sacrifice; (3) Ro4-4602 HCl (800 mg/kg, i.p.) 50 min or 90 min prior to sacrifice; and (4) Ro4-4602 (800 mg/kg, i.p.) preceding tryptophan (100 mg/kg) by 30 min. Animals were decapitated and their brains were rapidly removed, dissected, and frozen on solid CO_2. Forebrains and blocks of tissue containing raphe perikarya and proximal axons were dissected as described by AGHAJANIAN *et al.* (1973). Forebrains were homogenized in 4 volumes of 0·4 M perchloric acid and the blocks of midbrain tissue were homogenized in 4 ml of 0·4 M perchloric acid. Homogenates were used for assay of 5-HT by the method of SNYDER, AXELROD and ZWEIG (1965). Neither tryptophan nor Ro4-4602 were found to interfere with this assay. Recovery of 5-HT added to tissue was 80–85%.

Tryptophan levels in rat brain were also measured using the following combination of drugs: (1) saline; (2) tryptophan (100 or 200 mg/kg, i.p.) 30 min prior to sacrifice; (3) Ro4-4602 HCl (800 mg/kg, i.p.) 1 hr prior to sacrifice; and (4) Ro4-4602 HCl (800 mg/kg, i.p.) preceding tryptophan (either 100 or 200 mg/kg) by 30 min. Animals were decapitated and dissected as described above. Forebrains were homogenized in 4 vol. of 0·4 M perchloric acid and blocks of midbrain tissue were homogenized in 4 ml of 0·4 M perchloric acid. Homogenates were analyzed for tryptophan, using the column separation as described by LINDQVIST (1971) combined with the fluorometric assay of HESS and UDENFRIEND (1959). Recovery of tryptophan added to tissue was 65–75%.

Drugs

L-Tryptophan (10 mg/ml in distilled H_2O in a dose of 100 mg/kg, Calbiochem), L-5-hydroxytryptophan (15 mg/ml in H_2O in a dose of 150 mg/kg, Sigma Chemical Co.), Ro4-4602 [(N-DL Seryl)-N-(2,3,4-trihydroxybenzyl)hydrazine], HCl (150 mg/ml in H_2O in a dose of 800 mg/kg or 50 mg/ml in a dose of 50 mg/kg. Hoffman-LaRoche, Inc.) and *p*-chlorophenylalanine methyl ester (400 mg/kg, pretreatment 24–48 hr prior to recording, Regis Chemical Co.) were administered intraperitoneally. (+)-Lysergic acid diethylamide bitartrate (approximately 20 μg/kg when given) was administered intravenously. Statistical significance was calculated by Student's *t*-test (two-tailed).

RESULTS

The activity of single dorsal raphe cells was uniformly depressed within 5–10 min by the systemic administration of 100 mg/kg tryptophan (Fig. 1, Table 1). Treatment with Ro4-4602 (800 mg/kg, i.p.), an L-amino acid decarboxylase inhibitor, completely prevented the inhibition of raphe cell firing caused by systemically administered tryptophan (Table 1, Fig.

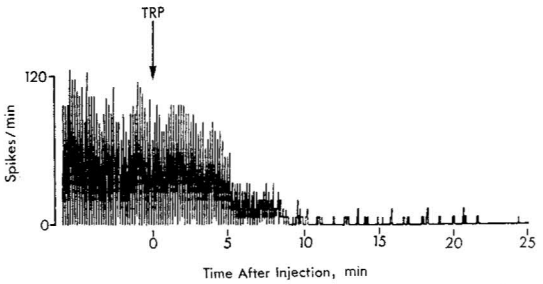

Fig. 1. Effect of a loading dose of tryptophan (TRP) on the rate of firing of a midbrain raphe neurone in the rat. Tryptophan (100 mg/kg, i.p.) was administered at time 0 and maximal inhibition reached after about 10 min.

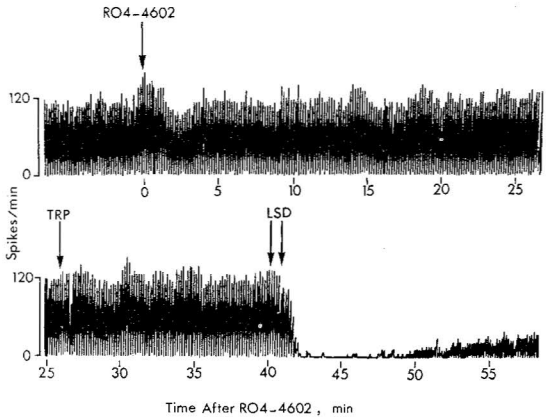

Fig. 2. A continous recording demonstrating the effect of Ro4-4602 pretreatment on the response of tryptophan in a dorsal raphe cell. Neither Ro4-4602 (800 mg/kg, i.p.) administered at time 0 nor tryptophan (TRP, 100 mg/kg, i.p.) given 26 min after Ro4-4602 affected the firing rate. However, LSD (total dose 20 μg/kg) was still effective in depressing firing despite the Ro4-4602 pretreatment.

2). Ro4-4602 (800 mg/kg, i.p.) given alone either caused no change or a slight acceleration of raphe firing rate (Table 1, Fig. 2). *p*-Chlorophenylalanine, a tryptophan hydroxylase inhibitor, did not block the inhibition of raphe firing by tryptophan (Table 1). Lysergic acid diethylamide (total dose 20 μg/kg) was effective in depressing raphe firing even when given after Ro4-4602 (Fig. 2).

As can be seen in Table 2, by 20 min Ro4-4602 significantly ($P < 0.001$) decreased the amount of endogenous 5-HT in both the raphe and forebrain areas even after tryptophan (100 mg/kg, i.p.) treatment; Ro4-4602 pretreatment uniformly prevented the tryptophan-induced increase in 5-HT. These data suggest that Ro4-4602 is capable of blocking the effect of tryptophan on raphe cell firing through an inhibition of the enzymatic formation of 5-HT. However,

Table 1. Response of raphe cells to the systemic administration of 5-HT precursors and drugs

Drug treatment*	No of cells tested	Effect of Firing Rate†		
		Depressed in 15 min	Accelerated in 15 min	No change in 15 min
Tryptophan	24	24	0	0
Ro4-4602	13	0	8§	5
Ro4-4602 + tryptophan	12	0	0	12
Tryptophan + *p*-CPA	8	8‖	0	0
5-HTP‡	6	6‖	0	0

* Tryptophan (100 mg/kg), Ro4-4602 (800 mg/kg) or 5-HTP (150 mg/kg) were administered intraperitoneally following a 5 min predrug recording. When both tryptophan and Ro4-4602 were used, Ro4-4602 was administered 30 min prior to tryptophan. *p*-CPA (400 mg/kg, i.p.) was given 48 hr prior to tryptophan.

† Predrug and 15 min post-drug raphe cell firing rates were measured for each drug treatment and compared for statistical significance using a paired *t*-test.

‡ Maximal depression of firing was achieved 30-40 min after 5-HTP in cells tested.

§ $P < 0.05$.

‖ $P < 0.001$.

Table 2. Effects of Ro4-4602 on 5-HT concentration (μg/g) in the midbrain raphe nuclei and the forebrain after tryptophan loading

*Time after tryptophan (min)	Brain area†	Drug tested			
		Control	Tryptophan	Ro4-4602	Tryptophan + Ro4-4602
20	Forebrain	0·40 ± 0·01	0·53 ± 0·02§	0·31 ± 0·01§	0·34 ± 0·02
	Raphe	2·22 ± 0·15	3·02 ± 0·21‡	0·99 ± 0·12§	1·03 ± 0·06§
60	Forebrain	0·43 ± 0·03	0·61 ± 0·06‡	0·30 ± 0·02‡	0·28 ± 0·03‡
	Raphe	2·30 ± 0·18	3·45 ± 0·22§	1·82 ± 0·38	2·22 ± 0·21

* Rats (n = 5) were given Ro4-4602 (800 mg/kg, i.p.) or saline 30 min prior to the administration of tryptophan (100 mg/kg, i.p.) or vehicle. Animals were sacrificed 20 or 60 min after the injection of tryptophan (or vehicle).

† Areas were dissected according to the method of AGHAJANIAN *et al.* (1973).

‡ $P < 0.02$.

§ $P < 0.001$.

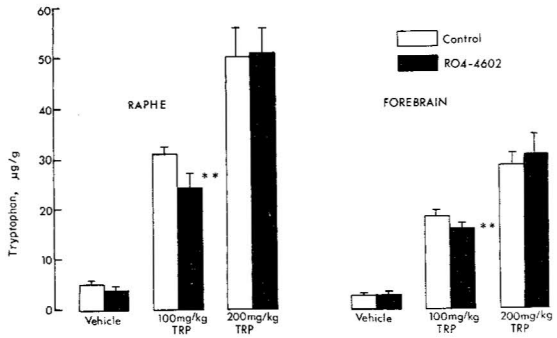

Fig. 3. A comparison of tryptophan levels in 2 brain areas after loading doses of tryptophan (TRP) and Ro4-4602 + TRP. Rats were injected with saline or Ro4-4602 30 min prior to the intraperitoneal injection of vehicle, or of 100 mg/kg or 200 mg/kg tryptophan. They were sacrificed 30 min after the tryptophan (or vehicle) injection. The dissection and assay of tryptophan in brain samples was as described in Methods. Results are expressed as mean ± S.D. (n = 4, $^{**}P < 0.02$) when compared with appropriate control value.

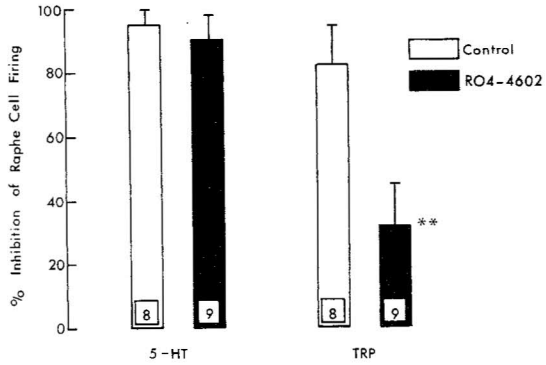

Fig. 5. Effect of Ro4-4602 pretreatment on the response of dorsal raphe cells to the microiontophoretic application of tryptophan (TRP) and 5-HT. This is a graphical presentation showing that while the sensitivity of dorsal raphe cells to 5-HT was not changed by Ro4-4602 (800 mg/kg, i.p.) pretreatment (9·5 ± 0·6 nA produced a mean inhibition of 95% vs. 8·9 ± 0·8 nA which produced a mean inhibition of 91%), the response of raphe cells to tryptophan was significantly reduced (103 ± 16 nA produces a mean inhibition of 82% vs. 102 ± 22 nA which produces a mean inhibition of 32%). Results are expressed as a percentage of the baseline firing rate ± S.D. ($^{**}P < 0.001$).

it is also possible that Ro4-4602 could act by inhibiting the uptake of tryptophan in brain, preventing its access to raphe cells. In fact, this appears to occur to some degree, since tryptophan levels in the raphe area were slightly but significantly lowered by both Ro4-4602 treatment alone, and by the combination of Ro4-4602 + 100 mg/kg tryptophan when compared to corresponding control values (Fig. 3). However, this effect could be overridden by a larger dose of tryptophan (200 mg/kg). Thus, it is significant that tryptophan in doses up to 300 mg/kg administered to rats pretreated with Ro4-4602, still failed to cause an inhibition of raphe firing (n = 4, not shown).

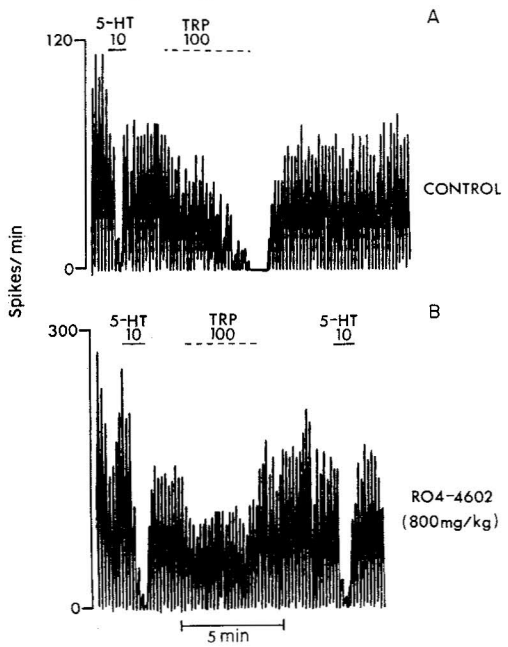

Fig. 4. Comparison of the sensitivity of raphe neurones to the microiontophoretic application of tryptophan (TRP) and 5-HT before and after systemic administration of Ro4-4602. In trace A (control) a raphe cell was completely inhibited by tryptophan (100 nA for 5 min) and by 5-HT (10 nA for 30 sec). In trace B, 30 min following the systemic administration of Ro4-4602 (800 mg/kg, i.p.) a raphe cell in the same animal was only partially inhibited by tryptophan (100 nA for 5 min). In contrast, even after Ro4-4602 pretreatment, 5-HT (10 nA for 30 sec) still produced a marked inhibition of raphe firing.

The microiontophoretic application of tryptophan for approximately 5 min at a mean ejection current of 103 ± 16 nA S.E.M. (n = 8) was found to gradually depress raphe firing until a mean inhibition of 82% of control firing was reached (Fig. 4A, Fig. 5). The ejection of 5-HT for 30 sec at a mean ejection current of 9·5 ± 0·6 nA S.E.M. (n = 8) resulted in a rapid inhibition of firing as previously reported (AGHAJANIAN *et al.*, 1972). Ejection of this amount of 5-HT was found to depress raphe firing by approximately 95% (Fig. 4A, Fig. 5). In contrast, current of 100 nA in the balance channel, resulting in the ejection of Na^+ ions, produced no effect on raphe firing even for periods greater than 5 min (n = 4, not shown). After pretreatment of animals with Ro4-4602 (800 mg/kg, i.p.), the response of raphe cell firing to the ejection of 5-HT (8·9 ± 0·8 nA S.E.M. for 30 sec) was essentially unchanged (approximately 91% inhibition; n = 9; Fig. 4B, Fig. 5). In contrast, the response of these same cells to the ejection of tryptophan (102 ± 22 nA S.E.M. for 5 min) was significantly attenuated by Ro4-4602 pretreatment (approximately 32% inhibition; $P < 0.001$ as compared with pre-Ro4-4602 inhibition by tryptophan; n = 9; Fig. 4B, Fig. 5). Although the depression of raphe firing by tryptophan administered systemically was completely blocked by Ro4-4602 pretreatment (Fig. 2), microiontophoretically applied tryptophan even after Ro4-4602 pretreatment was still found to produce a slight but significant ($P < 0.01$; n = 9) decrease in firing rate when compared with baseline firing rates (Fig. 4B).

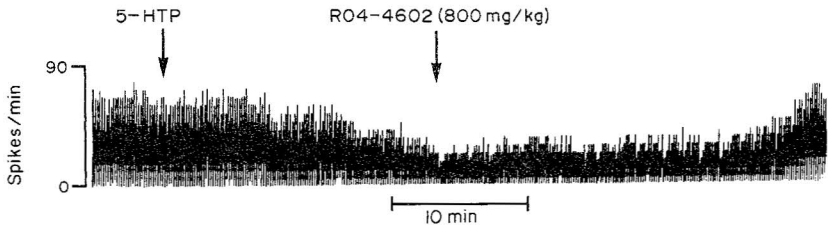

Fig. 6. Effect of 5-HTP on rate of firing of a midbrain raphe neurone. Peripheral decarboxylase was inhibited with Ro4-4602 50 mg/kg (i.p.) 30 min prior to the systemic administration of 5-HTP (150 mg/kg) as indicated by the arrow. Cessation of firing (not shown here) typically occurred approximately 30–40 min after 5-HTP. Recovery of firing was not observed, although some cells were monitored for up to 60 min after 5-HTP. Ro4-4602 (800 mg/kg i.p.) administered as indicated by the arrow, reversed the 5-HTP-induced depression within 20–25 min.

Following inhibition of peripheral decarboxylase by Ro4-4602 (50 mg/kg, i.p.), 5-HTP (150 mg/kg, i.p.) caused a gradual inhibition of raphe cell firing (Table 1). Maximum inhibition of raphe firing produced by 5-HTP was achieved after 30–40 min as compared with only 15–20 min for tryptophan. This inhibition of raphe firing by 5-HTP was reversed by Ro4-4602 at a dose of 800 mg/kg, i.p. (Fig. 6). In addition, the microiontophoretic application of 5-HTP for 3 min $\pm$ 19·4 sec at a mean ejection current of 25·0 $\pm$ 3·4 nA produced a gradual inhibition of raphe cell firing. In these same cells, the ejection of 5-HT for only 25 $\pm$ 2·2 sec at a current strength of 16·6 $\pm$ 2·1 nA produced a rapid inhibition of raphe cell firing (n = 9, not shown).

DISCUSSION

These studies show that the inhibition in raphe neuronal firing produced by the systemic administration of loading doses of the 5-HT precursors, tryptophan and 5-HTP, is blocked by Ro4-4602. The latter drug is an aromatic amino acid decarboxylase inhibitor which prevents the conversion of 5-HTP to 5-HT (BÉDARD *et al.*, 1971; CARLSSON and LINDQVIST, 1970). In the dose used, Ro4-4602 prevented the increase in 5-HT in both the raphe area and forebrain after the administration of a loading dose of tryptophan. This suggests that Ro4-4602 blocks the tryptophan-induced depression of raphe unit firing by preventing an accumulation of 5-HT. However, the possibility that the Ro4-4602 interfered with the tryptophan effect by blocking the uptake and/or accumulation of tryptophan in brain was also examined (CARLSSON and LINDQVIST, 1972). This mechanism was ruled out by observing that the levels of tryptophan achieved in brain following larger loading doses of tryptophan (200 mg/kg) were not affected by Ro4-4602 treatment. The failure of Ro4-4602 to block LSD supports the hypothesis that the LSD-induced depression of raphe firing is not dependent upon ongoing 5-HT synthesis (AGHAJANIAN *et al.*, 1970).

p-Chlorophenylalanine, another 5-HT synthesis inhibitor, also prevents an increase in 5-HT accumulation following a loading dose of tryptophan but only in the forebrain (AGHAJANIAN *et al.*, 1973). Furthermore, *p*-CPA was found to be ineffective in preventing the tryptophan-induced depression of raphe unit firing (AGHAJANIAN, 1972). Since one difference in the two synthesis inhibitors (*p*-CPA and Ro4-4602) is their efficacy in blocking 5-HT synthesis in the raphe region, the data suggest that the local increase in 5-HT in the vicinity of raphe perikarya after tryptophan loading is sufficient to produce the inhibition of raphe firing.

In order to explore further the possibility that tryptophan can inhibit raphe cell firing through its biochemical conversion to 5-HT within the raphe, both tryptophan and 5-HT were applied directly to raphe cells using microiontophoresis. In these experiments, dorsal raphe cells, which had the characteristics of identified serotonergic cells (AGHAJANIAN and HAIGLER, 1974), were found to be inhibited rapidly and uniformly by the microiontophoretic application of 5-HT as reported previously (AGHAJANIAN *et al.*, 1972; BRAMWELL and GONYE, 1973; HAIGLER and AGHAJANIAN, 1973). In addition, these cells were inhibited gradually by the direct ejection of tryptophan. The response of raphe cells to the microiontophoretic application of 5-HT was not affected by the pretreatment of the animal with Ro4-4602. In contrast, the effects of tryptophan applied iontophoretically were almost completely blocked by pretreatment with Ro4-4602. This data suggests that the effects of the amino acid tryptophan on the raphe are primarily due to its conversion to 5-HT within the raphe. In addition, the failure of Ro4-4602 to affect the response of raphe neurones to 5-HT applied microiontophoretically suggests that Ro4-4602 does not have any direct 5-HT blocking action. However, even after Ro4-4602, iontophoretically applied tryptophan was capable of producing a small but significant decrease in firing rate, suggesting that high local concentrations of tryptophan may have some direct cellular effects. The physiological significance of this observation is not clear, since Ro4-4602 was found to completely block the depression of raphe unit firing produced even by large (300 mg/kg) systemic doses of tryptophan. This occurred despite the fact that brain tryptophan concentration increases with increasing doses of systemically administered tryptophan (GUROFF and UDENFRIEND, 1962; GRAHAME-SMITH, 1971; CARLSSON and LINDQVIST, 1972).

To test further the possibility of an inverse relationship between raphe firing and 5-HT concentration, 5-HTP, the immediate precursor of 5-HT was given. 5-Hydroxytryptophan has been shown to enter the brain and to induce an increase in 5-HT levels (BOGDANSKI, WEISSBACK and UDENFRIEND, 1958). Previous data from this laboratory suggested that doses of 5-HTP up to 100 mg/kg (i.p.) have little effect on the firing of raphe units (AGHAJANIAN *et al.*, 1970). However, using 50 mg/kg Ro4-4602, a dose which blocks mainly peripheral decarboxylase (BARTHOLINI and PLETSCHER, 1968), 5-HTP (150 mg/kg, i.p.) was found to produce a gradual inhibition of raphe cell firing. This is in agreement with the recent results of TRULSON and JACOBS (1975). In contrast to tryptophan, whose effects on raphe firing were maximal by 15–20 min after administration, the inhibition induced by 5-HTP developed so slowly that 30–40 min was required to achieve a maximum reduction in raphe cell firing. The reason for such a gradual inhibition is not known. However, several studies have suggested that 5-HT synthesized in brain from exogenous 5-HTP may be formed mainly outside 5-HT-containing nerve endings. Thus MOIR and ECCLESTON (1968) found an abnormal regional distribution of 5-HT following the administration of 5-HTP. In addition, administration of high doses of 5-HTP along with peripheral decarboxylase inhibition resulted in indoleamine fluorescence inside dopaminergic and noradrenergic as well as serotonergic neurons (BUTCHER, ENGEL and FUXE, 1972). On the other hand, KORF, VENEMA and POSTEMA (1974) have suggested that 5-HTP in very low intravenous doses (12·5 mg/kg) combined with a peripheral decarboxylase inhibitor may be specifically decarboxylated in 5-HT-containing neurones. In these studies, 5-HTP appears to inhibit raphe cell firing through its biochemical conversion to 5-HT in the CNS, since administration of Ro4-4602, in a dose sufficient to block central decarboxylase activity (BÉDARD *et al.*, 1971), reversed the inhibitory effects of 5-HTP. 5-Hydroxytryptophan was also applied directly onto raphe cells via microiontophoresis. While 5-HTP produced a rapid inhibition of raphe cell firing 5-HTP ejected onto the same cells produced inhibition only after longer periods of ejection and slightly higher currents.

In conclusion, the present results are consistent with the hypothesized inverse relationship between serotonin accumulation in the raphe and the rate of raphe unit firing. Both the systemic and direct iontophoretic application of tryptophan, the initial precursor in the biosynthesis of brain serotonin, and of 5-HTP, the immediate precursor of 5-HT, inhibited raphe neuronal activity. By using Ro4-4602, a decarboxylase inhibitor which rapidly blocks the formation of 5-HT in raphe neurones, it was possible to show that both 5-HT precursors inhibit raphe firing through their enzymatic conversion to 5-HT. The ability of Ro4-4602 but not *p*-CPA to block the depression of raphe activity induced by tryptophan suggests that a local increase in 5-HT within the raphe perikarya as seen after *p*-CPA, is sufficient to decrease raphe cellular activity. Thus, in terms of a negative feedback hypothesis, firing rate adjustments could be determined by changes in local (raphe) 5-HT concentrations. It is possible to speculate that local increases in 5-HT could affect raphe firing by 2 mechanisms: (1) 5-HT could leak directly from the raphe soma to produce a local inhibitory effect; and/or (2) if there are collaterals from 5-HT neurones which terminate on other 5-HT cells, then an increase in 5-HT released from these terminals could result in a depression of raphe firing.

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