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AGN-2979, an inhibitor of tryptophan hydroxylase activation, does not affect serotonin synthesis in Flinders Sensitive Line rats, a rat model of depression, but produces a significant effect in Flinders Resistant Line rats

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Abstract

The neurotransmitter, serotonin, is involved in several brain functions, including both normal, physiological functions, and pathophysiological functions. Alterations in any of the normal parameters of serotonergic neurotransmission can produce several different psychiatric disorders, including major depression. In many instances, brain neurochemical variables are not able to be studied properly in humans, thus making the use of good animal models extremely valuable. One of these animal models is the Flinders Sensitive Line (FSL) of rats, which has face, predictive and constructive validities in relation to human depression. The objective of this study was to quantify the effect of the tryptophan hydroxylase (TPH) activation inhibitor, AGN-2979, on the FSL rats (rats with depression-like behaviour), and compare it to the effect on the Flinders Resistant Line (FRL) of rats used as the control rats. The effect was evaluated by measuring changes in regional serotonin synthesis in the vehicle treated rats (FSL-VEH and FRL-VEH) relative to those measured in the AGN-2979 treated rats (FSL-AGN and FRL-AGN). Regional serotonin synthesis was measured autoradiographically in more than thirty brain regions. The measurements were performed using α -[¹⁴C]methyl-L-tryptophan as the tracer. The results indicate that AGN-2979 did not produce a significant reduction of TPH activity in the AGN-2979 group relative to the vehicle group (a reduction would have been observed if there had been an activation of TPH by the experimental set up) in the FSL rats. On the other hand, there was a highly significant reduction of synthesis in the FRL rats treated by AGN-2979, relative to the vehicle group. Together, the results demonstrate that in the FSL rats, AGN-2979 does not affect serotonin synthesis. This suggests that there was no activation of TPH in the FSL rats during the experimental procedure, but such activation did occur in the FRL rats. Because of this finding, it could be hypothesised that TPH in the FSL rats cannot be easily activated. This may contribute to the development of depressive-like symptoms in the FSL rats (“depressed” rats), as they cannot easily modulate their need for elevated amounts of this

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neurotransmitter, and possibly other neurotransmitters. Further, because these rats represent a very good model of human depression, one can hypothesize that humans who do not have readily activated TPH may be more prone to develop depression.

Keywords

Autoradiography; serotonin; synthesis; tracer; tryptophan hydroxylase activation inhibitor

Introduction

5-hydroxytryptophan (serotonin; 5-HT) synthesis is one of the most important presynaptic parameters of serotonergic neurotransmission (Nelson, 1993). The study of synthesis in animal models, as well as humans, is very important in obtaining a better understanding of the deficiencies in affective disorders. Several neurochemical brain systems are involved in affective disorders, including glucose utilization (Mayberg 2003), 5-HT receptors (Drevets et al. 1999; Bhagwagar et al. 2006), 5-HT synthesis (Rosa-Neto et al. 2004; Berney et al. 2008) and 5-HT transporters (Firk and Markus, 2008; Ruhé et al. 2009). More recently, new hypotheses of neurotransmission deficiencies/alterations in depression have been proposed (Kiss, 2008). These could, in part, explain the creation of the non-physiological circuitry (*e.g.*, 5-HT could be released outside of the synapse) which may underlie the depressive state.

It has been shown that the activation of tryptophan hydroxylase (TPH; EC 1.14.16.4) can be prevented in stressful situations by the TPH activation inhibitor, AGN-2979 [3-(3-methoxyphenyl)-3-(3-dimethylaminopropyl)-4, 4-dimethylpiperidine-2, 6-dione] (Boadle-Biber et al. 1982; Boadle-Biber and Phan, 1986). AGN-2979 has also been shown to induce antidepressant activity (Boadle-Biber et al., 1989) in rats, and in a pilot clinical trial, the onset of antidepressant action of AGN 2979 was faster than other antidepressants (Clerc et al., 1986). These studies suggest that AGN-2979 induces antidepressant action.

The Flinders Sensitive Line of rats (FSL; “depressed” rats) and the Flinders Resistant Line (FRL) of rats were developed from Sprague-Dawley (SPD) rats through selective breeding (Russel et al., 1982). The FSL rats were selected for their high sensitivity to the cholinesterase inhibitor, diisopropyl fluorophosphates. The FRL rats showed low sensitivity to this inhibitor (Overstreet, 2002). This supersensitivity of the FSL rats has been linked to the cholinergic hypothesis of depression (Janowsky and Risch, 1984), as well as to the hypothesis regarding the serotonergic and cholinergic actions of antidepressants (Dilsaver, 1986; Janowsky and Risch, 1984; Plaznik et al. 1989). The FSL rats have been described as a rat model of human depression with psychomotor retardation (Overstreet et al. 2005), which is similar to a type of depression described in humans (*i.e.*, retarded depression - a state of clinical depression in which the individual is lethargic and slow to initiate action: Lapierre and Butter, 1980).

The FSL, or “depressed”, rats have many behavioral differences (*e.g.*, altered sleep patterns, increased total rapid eye movement, lower levels of activity, increased immobility in the Porsolt swim test, cognitive deficit, and “anhedonia” (as inferred from a sucrose consumption test) following chronic mild stress) and neurochemical differences (cholinergic, gabanergic, neurosteroid, dopaminergic, receptor (*e.g.*, 5-HT_{2C}, 5-HT₃, GABA_A), and differences in the Hypothalamic-Pituitary-Adrenal axis) when compared to either the SPD or FRL rats (reviewed in Yadiid et al. 2000 and Overstreet et al. 2005 and 2008). The FSL rats also have lower 5-HT synthesis than normal SPD or FRL rats (Hasegawa et al. 2006). In the FSL rats, 5-HT synthesis is acutely reduced by citalopram throughout the brain, while it is elevated in the FRL rats (Kanemaru et al. 2008). The FSL rats also have a lower density of 5-HT_{1A} receptors than the SPD or FRL rats (Nishi et al. 2009). However, the density of the 5-HT_{1B} receptors, which may

be important in the etiology of depression and the action of antidepressants, is significantly greater in the FSL rats than in the FRL and SPD rats (Nishi et al. 2009). The FSL rats have a greater tissue concentration of 5-HT in several brain regions than the SPD rats, but a lower 5-HT turnover, and both are normalized through chronic treatment with desipramine (Overstreet et al. 2005). In the FSL animal model of depression, the alleviation of depressive symptoms is achieved following chronic, but not acute, treatment with antidepressants (Yadid et al. 2000; Overstreet et al. 2008).

We recently demonstrated that in normal Sprague-Dawley (SPD) rats, an acute treatment of AGN 2979 produces a reduction in 5-HT synthesis relative to the synthesis in vehicle treated rats (Hasegawa et al. 2005). This observation was interpreted as AGN-2979 preventing TPH activation produced by the experimental setup (*e.g.*, stress) (Hasegawa et al. 2005).

Based on the known deficiencies found in the FSL rats, as briefly summarized above and referenced, we designed a study to answer the following questions: 1) Is TPH activated, as measured through 5-HT synthesis, by experimental procedure, and does the TPH activation inhibitor, AGN-2979, prevent activation in the FSL rats (“depressed” rats; when comparing the saline and AGN-2979 groups)? 2) Is the activation of TPH in the FSL rats different from that in the FRL rats? 3) How does the activation differ in these two strains of rats from that reported previously (historic controls) in normal SPD rats (Hasegawa et al. 2005), and are the effects different in different brain regions?

Material and Methods

All of the animals were housed in the animal facilities at the Montreal Neurological Institute with automatic light control (7 p.m. to 7 a.m. dark cycle). Both the FSL and FRL rats were obtained from breeding colonies maintained at the Montreal Neurological Institute from virus free breeds kindly supplied by Dr. D. Overstreet (Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599-7178, U.S.A.). All of the animal procedures were performed in strict accordance with the Canadian Council on Animal Care guidelines and were approved by the Animal Care Committee of McGill University. Before the experiment, the animals were fasted overnight (approximately 12 h) to stabilize the plasma glucose and amino acids, in particular, tryptophan and the amino acids sharing the same blood barrier carrier. Water was provided *ad libitum*. The tracer was always injected between 12:00 and 2:00 p.m., and the rats were euthanized between 2:00 and 4:00 p.m. The AGN-2979 was provided as a generous gift from Dr. MW Gittos. The dose of AGN-2979 was selected on the basis of the inhibition of the probenecid activated 5-HT turnover data (Boadle-Biber, 1982), and the drug was dissolved in deionized water. There were fourteen rats in the FSL-VEH group, seventeen in the FSL-AGN group, and eleven in each of the FRL-VEH and FRL-AGN groups. The rats within each strain were randomly assigned to groups. In these experiments, there were two groups of FSL rats (FSL vehicle and AGN-2979 treated; FSL-VEH and FSL-AGN, respectively) and two groups of FRL rats (FRL vehicle and AGN-2979 treated; FRL-VEH and FRL-AGN, respectively). On the experiment day, the arterial and venous catheters (for blood sampling and tracer injection, respectively) were implanted under 1% – 1.5% isoflurane anesthesia. The lower part of the rat body was placed in a loose-fitting plaster cast, and the rats were allowed to awaken. One hour later, a solution of AGN-2979 (10 mg/kg; i.p) or the vehicle was injected as a volume of 1 ml/kg. The timing of the drug and tracer injections were the same as those used in the previous study (Hasegawa et al. 2005), and were selected based on information provided by Dr. M.W. Gittos (personal communication). All of the surgical procedures performed have been described in detail in previous publications (Nagahiro et al. 1990; Kanemaru et al. 2008), and only a brief description will be provided here.

Approximately 30 μCi of α -[^{14}C]methyl-L-tryptophan (α -[^{14}C]MTrp; the tracer was obtained from Amersham Biosciences and had specific activity of about 50 mCi/mmol) over a period of two minutes was injected into each rat one hour following the drug or vehicle injection. This was more than two hours after surgery. An injection over a 2 min period was done to reduce the danger of altering the steady-state tryptophan metabolism (see details in Nagahiro et al. 1990; Diksic et al. 1990 and 1995; Tohyama et al. 2002). Approximately 50 μl of blood (20 μl plasma used for liquid scintillation measurements) was taken every 20 seconds for the first two minutes and then at progressively increased intervals to obtain a good input function, until euthanasia by guillotine. All of the blood withdrawn was replaced with saline following each sampling. A total of approximately 0.7 mL of blood was taken during the experimental procedures. Blood pO_2 , pCO_2 , pH, and hematocrit levels were checked periodically to ensure a physiologically stable animal. Twelve to fourteen plasma samples were taken from the beginning of the injection until the rats were euthanized. Five additional plasma samples for the determination of the plasma tryptophan concentration (free in ultrafiltrate with 10000 MW cut off and total following deprotonization with 20% trichloroacetic acid) were taken at different times. The rats were euthanized at 60 and 150 min (approximately half of the rats each time) from the beginning of the tracer infusion. These two points at which the rats were euthanized were selected based on the measurements of the brain uptake and trapping of the α -[^{14}C]MTrp tracer (Diksic et al. 1990 and 1995; Diksic and Young, 2001). The first point (60 min) is the time at which the tracer reaches a more or less apparent steady state (Diksic et al. 1990), while 150 min is a compromise between the length of experiment (rat immobilization) and the tracer kinetics (uptake/trapping). Between these two time points, the uptake of the tracer can be approximated by a straight line which represents the rate of uptake (Nagahiro et al. 1990; Diksic et al. 1995), and can be converted into the 5-HT synthesis rate (Diksic and Young, 2001). The tracer uptake is modeled as a three compartment and three rate constant biological model (Diksic et al. 1990; Diksic and Young, 2001).

The brain was removed as soon as possible (usually within one minute) and frozen by immersion into cold (-20°C) iso-pentane. The brains were cut into 30 μm sections in the microtome at -20°C and dried on a hotplate at 60°C . The sections, along with the standards calibrated in nCi per g-brain tissue, were placed for 3 weeks against X-ray film and developed. Autoradiograms were digitized using a computerized video digitizer (MCID-4; Imaging Research Inc. St. Catharines, Ont. Canada). The brain regions identification were done by an aid of a rat atlas (Paxinos and Watson, 1997). The readings from the left and right sides were averaged (there was no significant left-to-right differences) and the value obtained was used as representative for a particular brain region. All regions were read from 3–4 consecutive cross sections. After conversion into the volume of distribution (VD [ml/g]; division of tissue radioactivity by plasma tracer concentration (Cp ; nCi/ml) at the time of euthanasia), the slope of the unidirectional net uptake of the tracer, required in the rate calculation, was calculated from the linear relationship between VD (ml/g) and exposure time Θ ($\Theta = [\text{Cp} \cdot \text{dt} / \text{Cp}(T)]$ [min] (see details in Nagahiro et al. 1990; Diksic et al. 1995; Diksic and Young, 2001). The LC of 0.42 measured in the rats was used in the calculations (Vanier et al. 1995).

In the Discussion section, a comparison of the effect in the historic control group (normal SPD rats; Hasegawa et al. 2005) was performed to obtain a better understanding of the present results.

Statistical Evaluation

A General Linear Model (GLM) ANOVA was used to determine if there are differences between the saline treated group (the control) and the AGN-2979 treated group of rats, as well as if there is a significant interaction between the different groups and the brain regions among these groups. A planned comparison was used as a post-hoc evaluation of possible differences

and they were always done between the treatment group and its respective control group (e.g., FSL vehicle relative to FSL AGN-2979). To determine which of the brain regions were different in any of the sets, one-way ANOVA was calculated for each comparison between the control and respective treatment group as part of the planned comparison evaluations. In addition, the False Discovery Rate Correction (FDR), valid for samples with significant correlations, was applied (Benjamini and Yekutieli, 2001). However, this correction may not be essential when a planned comparison is used and the number of comparisons is less than the number of degrees of freedom. $p < 0.05$ was taken to indicate significance. In addition some authors (e.g. Rothman, 1990) argue that the underlining base for doing adjustment for multiple tests is the assumption that chance is the most prevalent explanation for an association between variables. However this is probably not correct because the nature is governed by physical laws and not chance. Note that in all of the experiments, the vehicle treated groups were compared to the AGN-2979 treated groups, and the difference between them indicated an effect of AGN-2979. There were no formal comparisons made between the FSL-VEH and FRL-VEH groups because the objective was to evaluate drug action and there is a possibility that the experimental setup (*i.e.*, saline injection) has a somewhat different, direct and/or indirect, effect on regional 5-HT synthesis in these two strains. Further, for the purpose of this discussion, formal statistical comparisons were made with the SPD results reported previously (Hasegawa et al. 2005). For these comparisons, all of the brain regions in which AGN-2979 produces a significant effect in the FRL rats were selected. All of the statistical evaluations were performed with Statistica 7.1 (StatSoft Inc., USA).

Results

The physiological parameters of rats used in the described experiments were within the values found in other experiments in the laboratory (Hasegawa et al. 2006; Kanemaru 2008 and 2009). The weights [g] of the rats were: 213.6 ± 2.9 (FRL-VEH); 212.5 ± 2.1 (FRL-AGN); 210.6 ± 2.7 (FSL-VEH); and 212.4 ± 2.1 (FSL-AGN). The plasma concentrations [nmol/mL] of tryptophan were: 7.5 ± 2.2 (free) and 59.1 ± 13.8 (total) for FRL-VEH rats; 7.4 ± 2.5 (free) and 65.2 ± 16.3 (total) for FRL-AGN rats; 8.6 ± 2.5 (free) and $64. \pm 12.8$ (total) for FSL-VEH rats; 7.5 ± 2.4 (free) and 60.6 ± 13.7 (total) for FSL-AGN rats. A comparison of all of the physiological parameters revealed no significant differences ($F(1,29)=0.17$; $p > 0.5$ for FSL, and $F(1,20)=0.33$; $p > 0.5$ for FRL groups) between the AGN-2979 group and the respective vehicle group in any of the strains of rats.

A set of autoradiographic images depicting regional variability of 5-HT synthesis in the FSL and FRL rats treated with either saline or AGN-2979, the inhibitor of TPH activation, is provided in Fig. 1. The images clearly show that 5-HT synthesis is not uniform throughout the rat brain, and that some structures, such as the caudate and cortex, have more pronounced non-homogeneity. It can also be seen that the synthesis is substantially greater in the pineal body and raphe nuclei than in other parts of the brain. Quantitative values of regional synthesis are provided in Table 1 for the vehicle and AGN-2979 treated FSL and FRL rats.

The ANOVA analysis revealed no significant difference ($F(1,29)=0.52$; $p > 0.4$) in 5-HT synthesis between the FSL-VEH and FSL-AGN groups. The interaction between the groups and the brain regions was significant (group*region interaction $F(34,986)=22.1$; $p < 0.001$), which prompted a planned *post hoc* evaluation of the synthesis between the groups in each of the regions. The planned comparison revealed significant ($p < 0.05$) differences in the auditory cortex ($F(1,29)=5.4$; $p < 0.03$), ventral hippocampus ($F(1,29)=6.2$; $p < 0.02$), hypothalamus ($F(1,29)=5.9$; $p < 0.03$), and dorsal raphe ($F(1,29)=19.0$; $p < 0.001$). Using FDR as a correction for multiple tests, significance was only revealed in the dorsal raphe. There was no effect on the synthesis in the pineal body.

A comparison between the FRL groups revealed a significant difference ($F(1,20)=7.1$; $p<0.02$) between the FRL-VEH and FRL-AGN groups, with significant interaction (group*region interaction $F(34,680)=55.7$; $p<0.001$). A planned *post hoc* analysis revealed significant differences in twenty-four out of thirty-five brain regions evaluated (Table 1). However, after performing FDR, the significance was lost in seven brain regions (CxP, CxF, Scn, LG, AN, CPI, and SNc). It is interesting to note that in the FRL rats, the AGN-2979 did not produce a significant effect in the synthesis between the FRL-VEH and FRL-AGN groups in the CxA, HiV and Hyp, the regions which showed differences between the FSL-VEH and FSL-AGN groups (see above and Table 1). The AGN-2979 treatment that produced the largest effect in the FRL rats was in some brain limbic structures (*e.g.*, Amy, AONu, CxCin and CxEnt). There was no effect observed in the pineal body, which is not surprising, given that the pineal body has a TPH-1 isoform (McKinney et al. 2005).

Discussion

The most important finding described here is the inability of the TPH activation inhibitor, AGN-2979, to influence 5-HT synthesis in the terminal regions of FSL rats, while the same treatment of the FRL rats under the same experimental conditions produced a significant reduction in synthesis (Table 1). It is interesting to note that there was a significant activation of TPH by the experimental procedure used in the DR, the cell body region in both the FSL and FRL rats (Table 1). These observations suggest that the experimental setup used produced an activation of TPH in the FRL rats (the activation of TPH is inhibited in the AGN group by AGN-2979) but there was no activation in the FSL rats, except in the dorsal raphe. This further indicates that TPH in the brain of the FSL rats is not easily activated, suggesting that the drug concentration in the brain of both strains was at pharmacological levels. This is a reasonable assumption because the effect of AGN-2979 was observed in the DR of both the FSL and FRL rats but not in the terminals of the FSL rats. A similar effect of AGN-2979 to that observed in the FRL rats has been reported in the treatment of normal SPD rats with AGN-2979 and under an identical experimental protocol (Hasegawa et al. 2005).

It is also interesting that there was a significant effect in many limbic structures (*e.g.*, CxCin, CxEnt, Amy, HiD) and cell body structures (*e.g.*, DR and MR) in the FRL rats (present study; Table 1), which is similar to the effect previously reported in the SPD rats (Hasegawa et al. 2005), but not in the FSL rats, apart from the DR (Table 1). These results suggest two possibilities: there are differences in processes of TPH activation in the DR and terminal regions in the FSL rats, or the processes which lead to the activation of TPH in the terminal areas require a longer period of time to be observed. A graphical comparison of the effects in the selected brain regions shown in Fig. 2 demonstrates that the inhibitory effect of AGN-2979 on TPH activation is present throughout the brain in the FRL rats (reduction of synthesis in treated rats), with little effect in the FSL rats. Further, the inhibitory effect of AGN-2979 on the activation of TPH in the SPD rats is approximately the same as that obtained in the FRL rats (a formal ANOVA test showed no difference in the effect between these two strains; $F(1,71)=0.01$; $p>0.5$). This modulation of 5-HT synthesis present in the FRL and SPD rats in the limbic structures (as shown in Table 1 and Fig. 2) could be important because depressed humans have lower 5-HT synthesis in the cingulate (Rosa-Neto et al. 2004), which is elevated by antidepressant treatments (Berney et al. 2008).

The exact mode of action (the way in which AGN-2979 prevents the activation of TPH) of AGN-2979 is not known, but it could be hypothesized that the FSL rats' TPH cannot be easily activated (possibly due to a number of factors, including phosphorylation, conformational change, or the influx of calcium). There is some experimental evidence suggesting that AGN-2979 works, in part, by blocking K⁺ channels (Gittos and Papp, 2001; Boadle-Biber and Phan, 1986), the mechanism shared by several antidepressants (Yeung et al., 1999 and 1999;

Choi et al., 1999). Because the ion channels are coupled with 5-HT receptors (Nicoll, 1988; Hille, 1992), direct or indirect agonists (such as selective serotonin uptake inhibitors) could act on the ion channels and subsequently on 5-HT synthesis by controlling TPH activity (Yeung et al., 1999 and 1999; Choi et al., 1999) and possibly the influx of Ca^{2+} , which can also modulate TPH activity.

There have been many investigations of the mechanisms by which antidepressants work, but we still do not have a clear understanding of these mechanisms. Recent proposals of the mechanism of action suggest the interaction of antidepressants with K^+ channels. Based on the findings that many antidepressants block K^+ channels (Sung et al. 2008; Ohno et al. 2007; Wooltorton and Mathie, 1993; Yeung et al., 1999; Choi et al., 1999), the blockade of neuronal K^+ -channels has been proposed as part of a mechanism for AGN 2979 antidepressant-like action (Gittos and Papp, 2001). Similarly, the selective serotonin reuptake inhibitors, paroxetine, sertraline, and fluoxetine, have potent K^+ -channel blocking properties (Velasco et al., 1995; Yeung et al., 1999), similar to tricyclic anti-depressants (Wooltorton and Mathie, 1993).

If parallels are drawn between the results in the FSL rats (in addition to the many other neurochemical differences mentioned above the FSL rats have limited ability to modulate TPH activity as measured by 5-HT synthesis), the rat model of depression, and humans with depression, one can hypothesize that depressed humans have a limited ability to modulate 5-HT synthesis which could be a factor in the etiology of depression, as well as its treatment and relapse incidence. This hypothesis is also supported by the fact that acute tryptophan depletion produces a relapse in treated depressed patients (Delgado et al. 1991) who have regionally lower 5-HT synthesis (Rosa-Neto et al. 2004), which suggests that the activity of TPH cannot be properly modulated to synthesize required amounts of 5-HT.

It has previously been shown that stress can cause the activation of TPH (Boadle-Biber et al. 1989 and 1983), and can also trigger a rather complex chain of events which, in some cases, can lead to depression (Frank et al. 1990; Kessler et al. 1994; Gold et al. 1988). It has also been shown that stress produces the activation of TPH which can be blocked by AGN-2979 (Gittos and Papp, 2001). Finally, some studies have demonstrated that AGN-2979 has some antidepressant-like activity (Boadle-Biber et al., 1989; Gittos and Papp, 2001) and produces a significant improvement in patients with generalized anxiety disorder (Cutlet et al., 1994).

As the FSL rats cannot increase the activity of TPH and 5-HT synthesis (based on the present results) in somewhat stressful situations of the present experimental setup, and because greater amounts of 5-HT are released in some brain regions during stressful situations (Chaouloff, 1993), the 5-HT neuronal releasable pool becomes depleted of 5-HT. This depletion of the 5-HT releasable pool can be detrimental to the development and maintenance of normal neuronal circuitry, especially during the developmental stage of the brain, as 5-HT is one of the known trophic factors. During this stage, stressful situations occur and they can create, along with the above mentioned deficiency, non-physiological circuitry in FSL rats, which could contribute to their depressive-like behaviour.

In conclusion, it could be stated that the results presented here (Table 1), together with those reported previously on normal SPD rats, suggest that FSL rats, a genetic rat model of depression, do not easily activate TPH, while normal SPD and FRL (“non-depressed”) rats do. This non-existent reserve of TPH, which could normally be modulated as required by different situations (*e.g.*, stress), could be, in part, responsible for the pathophysiological state found in depressed subjects.

Abbreviations

α -[^{14}C]MTTrp, α -[^{14}C]methyl-L-tryptophan
 AGN-2979, 3-(3-methoxyphenyl)-3-(3-dimethylaminopropyl)-4, 4-dimethylpiperidine-2, 6-dione
 AMY, amygdala
 AN, accumbens
 AONuc, anterior olfactory nucleus
 Cl, claustrum
 CN, caudate nucleus
 CPl, caudate-putamen (lateral)
 CPm, caudate-putamen (median)
 CxA, auditory cortex
 CxCin, cingulate cortex
 CxEn, entorhinal cortex
 CxF, frontal cortex
 CxP, parietal cortex
 CxSM, somatosensory cortex
 CxV, visual cortex
 DR, dorsal raphe
 DS, dorsal subiculum
 FDR, False Discovery Rate Correction
 FRL-AGN, FRL-AGN-2979
 FRL, FlindersResistant Line
 FRL-VEH, FRL-vehicle
 FSL-AGN, FSL-AGN-2979
 FSL, Flinders Sensitive Line
 FSL-VEH, FSL-vehicle
 GLM, General Linear Model
 ANOVA, analysis of variance
 GP, globus pallidus
 HiD, hippocampus (dorsal)
 HiV, hippocampus (ventral)
 Hyp, hypothalamus
 LC, locus coeruleus
 LG, lateral geniculates
 MBF, median forebrain bundle
 MG, medina_geniculate
 MR, median raphe
 PB, pineal body
 RM, raphe magnus
 Rpo, pontine raphe
 SC, superior colliculus
 Scn, suprachiasmatic Nucleus
 Sep.n., septal nucleus
 SNc, substantia nigra compacta
 SNr, substantia nigra reticulata
 SPD, Sprague-Dawleys
 ThD, thalamus (dorsal)
 ThV, thalamus (ventral)
 TPH, tryptophan hydroxylase
 VTA, ventral tegmental area

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Figure 1.

A set of autoradiograms exemplifying regional differences between 5-HT synthesis in the FSL and FRL rats. The control rats were treated with a vehicle (FSL-VEH and FRL-VEH) while the treatment group was treated with 10 mg/kg of AGN-2979. The meanings of the abbreviations are provided in the list of abbreviations.

Figure 2.

The changes in regional synthesis were calculated relative to the synthesis in the control treated rats for the brain regions in which significant effects were observed in the FRL rats. The error bars are SEM. The bar graph clearly depicts the variability of the effect of the AGN-2979 treatment in the FRL rats and a lack of significant effect in the FSL rats. In addition, it shows the similarity of the effects in the FRL and normal SPD rats. The values of the x-axis correspond to brain regions as follows: 1=CxCin; 2=CxEnt; 3=CxF; 4=CxP; 5=CL; 6=AMY; 7=AN; 8=AONu; 9=DS; 10=HiD; 11=DR; 12=MR; 13=Rpo; 14=CPL; 15=GP; 16=SNc; 17=SNr; 18=ThD; 20=ThV; 21=LG; 22=MBF. The meanings of these abbreviations are provided in the list of abbreviations. The symbol * indicates the regions in which AGN-2979 produced a significant effect in FRL rats following FDR correction. The effects in all of the brain regions of SPD rats, used as comparisons, were significant (Hasegawa et al. 2005).

Table 1

Regional 5-HT synthesis (pmol/g/min), given as the mean $\pm$ SEM, for groups of FSL and FRL rats treated with AGN-2979 (10 mg/kg; I.P.), and the respective controls treated with saline. Injections were done one hour before the tracer injection.

Brain Region	Vehicle (N=14) ²	FSL Treatment (N=17) ²	Vehicle (N=11) ²	FRL Treatment(N=11) ²
Cortex				
Cx	24.0 $\pm$ 0.9	19.1 $\pm$ 1.8	20.2 $\pm$ 1.8	19.4 $\pm$ 1.3
Cin	22.4 $\pm$ 0.8	23.9 $\pm$ 1.3	21.1 $\pm$ 1.8 [†]	12.4 $\pm$ 1.4
Ent	30.6 $\pm$ 0.8	27.5 $\pm$ 1.8	32.6 $\pm$ 2.1 [†]	23.0 $\pm$ 1.4
F	19.5 $\pm$ 0.8	22.5 $\pm$ 1.5	19.9 $\pm$ 1.6 [‡]	15.2 $\pm$ 1.5
P	19.0 $\pm$ 0.8	21.0 $\pm$ 1.9	20.7 $\pm$ 1.8 [‡]	14.4 $\pm$ 1.5
SM	20.0 $\pm$ 0.8	22.6 $\pm$ 1.6	17.7 $\pm$ 1.8	15.8 $\pm$ 1.4
V	24.0 $\pm$ 0.8	22.4 $\pm$ 1.8	22.0 $\pm$ 1.9	17.2 $\pm$ 1.4
CI	29.7 $\pm$ 0.7	31.9 $\pm$ 1.5	28.4 $\pm$ 1.7 [†]	19.8 $\pm$ 1.2
Limbic area				
AMY	30.6 $\pm$ 0.6	27.3 $\pm$ 1.8	29.9 $\pm$ 1.7 [†]	19.8 $\pm$ 1.3
AN	38.4 $\pm$ 1.0	35.0 $\pm$ 1.9	37.4 $\pm$ 2.3 [‡]	31.4 $\pm$ 1.5
AONu	24.4 $\pm$ 0.6	24.9 $\pm$ 1.6	33.8 $\pm$ 2.3 [†]	21.5 $\pm$ 1.2
DS	21.5 $\pm$ 1.1	17.9 $\pm$ 1.7	20.9 $\pm$ 1.5 [†]	14.1 $\pm$ 1.4
HiD	28.7 $\pm$ 0.7	26.8 $\pm$ 1.8	31.0 $\pm$ 1.7 [†]	20.3 $\pm$ 1.2
HiV	33.9 $\pm$ 1.0	27.1 $\pm$ 2.3	28.2 $\pm$ 1.9	29.8 $\pm$ 1.7
Sep.n	28.8 $\pm$ 1.0	25.8 $\pm$ 1.7	25.7 $\pm$ 1.7 [†]	17.9 $\pm$ 1.5
Brain stem				
DR	127.3 $\pm$ 5.5 [#]	90.6 $\pm$ 6.1	102.1 $\pm$ 3.0 [†]	87.2 $\pm$ 1.4
LC	24.2 $\pm$ 0.7	20.2 $\pm$ 2.3	16.0 $\pm$ 2.3	16.0 $\pm$ 2.0
MR	72.6 $\pm$ 5.7	65.9 $\pm$ 4.8	78.4 $\pm$ 3.2 [†]	65.1 $\pm$ 1.8
RM	38.4 $\pm$ 1.6	40.7 $\pm$ 2.2	37.7 $\pm$ 2.1	36.1 $\pm$ 2.2
Rpo	34.1 $\pm$ 0.9	36.3 $\pm$ 2.1	40.3 $\pm$ 1.9 [†]	30.8 $\pm$ 1.4
SC	19.7 $\pm$ 0.6	21.0 $\pm$ 2.0	14.7 $\pm$ 1.7	15.7 $\pm$ 1.7
VTA	24.8 $\pm$ 0.6	23.2 $\pm$ 1.7	24.2 $\pm$ 1.8	19.7 $\pm$ 1.4
Basal ganglia				
CN	26.4 $\pm$ 0.7	27.4 $\pm$ 1.9	29.7 $\pm$ 1.8 [†]	20.5 $\pm$ 1.5
CPI	29.2 $\pm$ 0.9	25.2 $\pm$ 1.6	25.8 $\pm$ 1.6 [‡]	21.2 $\pm$ 1.4
CPm	31.7 $\pm$ 0.6	31.4 $\pm$ 2.1	29.2 $\pm$ 2.1	23.7 $\pm$ 1.7
GP	24.8 $\pm$ 0.9	23.0 $\pm$ 1.6	28.9 $\pm$ 1.7 [†]	16.5 $\pm$ 1.3
SNc	20.3 $\pm$ 0.5	19.5 $\pm$ 1.4	18.5 $\pm$ 1.6 [‡]	13.7 $\pm$ 1.6
SNr	18.4 $\pm$ 0.8	17.2 $\pm$ 1.3	18.2 $\pm$ 1.5 [†]	11.9 $\pm$ 1.4
Hypothalamus				
Hyp	29.4 $\pm$ 1.0	36.5 $\pm$ 2.5	30.4 $\pm$ 1.9	23.7 $\pm$ 2.7
Scn	32.0 $\pm$ 1.2	38.5 $\pm$ 3.1	40.0 $\pm$ 3.3 [‡]	29.3 $\pm$ 2.4
Thalamus				
ThD	20.9 $\pm$ 0.8	20.7 $\pm$ 1.8	19.4 $\pm$ 1.7 [†]	12.5 $\pm$ 1.5
ThV	20.8 $\pm$ 0.9	19.9 $\pm$ 1.9	20.2 $\pm$ 1.8 [†]	11.1 $\pm$ 1.7
LG	24.3 $\pm$ 0.8	22.1 $\pm$ 1.8	22.7 $\pm$ 1.9 [‡]	17.0 $\pm$ 1.6
MG	25.0 $\pm$ 0.6	22.8 $\pm$ 1.8	19.6 $\pm$ 1.7	16.4 $\pm$ 1.6
MBF	22.2 $\pm$ 0.9	21.1 $\pm$ 2.2	23.4 $\pm$ 1.8 [†]	15.3 $\pm$ 1.8
PB	599 $\pm$ 10	620 $\pm$ 10	654 $\pm$ 12	681 $\pm$ 15

¹The meanings of these abbreviations are provided in the list of abbreviations.

²N identifies the number of rats in the group

[#] Significant difference from the value in the AGN-2979 FSL treated rats following FDR correction.

[†] These values were significant from those in the AGN-2979 treated FRL rats following FDR correction at $p < 0.05$.

[‡] Comparing these values with those in the AGN-2979 treatment group ($p < 0.05$), but following FDR, the significance was lost