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Tryptophan, serotonin and dog behavior

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Dog trainer free lance.

Abstract: Serotonin, also known as 5-hydroxytryptamine (5-HT), has been firstly isolated and described by Maurice Rapport and colleagues, after decades of research on a vasoconstrictor substance suspected of being contained in platelets. After its discovery, several studies have been done to identify its location and function and 5-HT was quickly identified in many tissues, including the brain, lungs, kidneys, platelets and gastrointestinal tract. In addition to its role in platelet function, Brodie & Shore (1957), firstly proposed a role of 5-HT as a neurotransmitter, based on the localization of 5-HT receptors in specific areas of the vertebrate brain. The link between TRP metabolism, serotonin and behavior has been the subject of considerable interest and discussion in the dog. The use of TRP in the behavioral clinic of the dog is still controversial. While there is evidence of efficacy of products containing TRP and other sub-stances during anxiety syndrome and stress, the use of only TRP in the control of canine aggression has given, until now, inconclusive results.

Key Words: dog, behavior, serotonin, L-tryptophan.

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Introduction

Serotonin, also known as 5-hydroxytryptamine (5-HT), has been firstly isolated and described by Maurice Rapport and colleagues (Rapport et al., 1948), after decades of research on a vasoconstrictor substance suspected of being contained in platelets (Reid & Brick, 1942; Zucker, 1944). After its discovery, several studies have been done to identify its location and function and 5-HT was quickly identified in many tissues, including the brain, lungs, kidneys, platelets and gastrointestinal tract. In addition to its role in platelet function, Brodie & Shore (1957), firstly proposed a role of 5-HT as a neurotransmitter, based on the localization of 5-HT receptors in specific areas of the vertebrate brain (Twarog & Page, 1952; Amin et al., 1954).

Serotonin is produced in two phases. In the first phase, the essential amino acid L-tryptophan (TRP) is hydroxylated by the enzyme tryptophan hydroxylase (TPH) to 5-hydroxytryptophan (5-HTP).

Unlike other amino acids, TRP circulates in the blood and plasma mainly bound to albumin (Pardridge, 1979); only 10-20% of TRP is present as a free form in the plasma and there is a balance between the forms linked to albumin and the free forms in the peripheral circulation (McMenamy, 1965). At rest, approximately 90% of total plasma TRP is bound to albumin, forming a complex that cannot cross the blood brain barrier (BBB); the remainder circulates in a free form which is available for transport through the BBB in the brain (Madras et al., 1974). A determining factor in the relationship between free TRP and albumin-bound tryptophan is the plasma concentration of fatty acids non esterified (NEFA), which bind to albumin, displacing TRP from its binding site (Curzon et al., 1973), causing an increase in free TRP in plasma (Chaouloff et al., 1986; Blomstrand et al., 1989). Other exogenous factors can also divide TRP from albumin, as

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is the case with some drugs (Spano et al., 1974; Muller et al., 1975). It remains debated whether the binding of tryptophan with albumin can modify the availability of TRP for tissue metabolism (Smith & Pogson, 1980; Pardridge, 1983).

In a second step, 5-HTP is decarboxylated to form 5-HT (Mohammad-Zadeh et al., 2008). Studies by Clark and colleagues (1954) have shown that hydroxylation and decarboxylation occur almost instantly in the presence of TRP. Although both enzymes are necessary for the conversion of TRP into 5-HT, tryptophan hydroxylase is considered the rate limiting enzyme for several reasons; in fact, it has a relatively high Km and little affinity for other amino acids (Noguchi et al., 1973) and its distribution is limited to those tissues containing serotonin (Noguchi et al., 1973; Champier et al., 1997). The activity of this enzyme is in contrast with the non-specific enzymatic activity of tryptophan carboxylase, an enzyme that has an affinity for many amino acids. The latter is present in most tissues (Clark et al., 1954) and, since it is not a limiting factor in 5-HT synthesis, it is difficult to reduce neurotransmitter levels by inhibiting this enzyme (Mohammad-Zadeh et al., 2008).

In the Central Nervous System (CNS), 5-HT is synthesized and stored in presynaptic neurons, while, outside of it, the synthesis is limited to enterochromaffin cells and, to a lesser extent, to platelets.

The platelets may have a very limited capacity to produce 5-HT, however they represent an important storage site outside the CNS, because they readily absorb 5-TH from plasma (Toh, 1954; Hardisty & Stacey, 1955). 99% of the total body 5-HT has intracellular localization, which implies strict regulation (Mohammad-Zadeh et al., 2008). Its concentration in the tissues depends on the speed of synthesis and metabolism (Tyce, 1990). The primary metabolic pathway of serotonin is that of monoamine oxidase (MAO), which occurs in the cytosol of the neuron (McIsaac & Page, 1959). MAOs are ubiquitous enzymes, belonging to the class of oxidoreductases, which exist in two main forms: MAO-A and MAO-B. Serotonin is mainly inactivated by MAO-A (Sandler et al., 1981).

TRP, 5-HT and behavior

The link between TRP metabolism, serotonin and behavior has been the subject of considerable interest and discussion in the dog.

The first research dates to 1996 (Dodman et al., 1996) when it was determined the effect of diets containing a low, medium, or high protein content would have on behavior of dogs with dominance aggression, hyperactivity, territorial aggression or without behavioral problems. The results showed that the behavior of the animals with dominance aggression, with hyperactivity and that of control dogs was unchanged by the dietary manipulations. Instead, territorial aggression was significantly reduced when dogs were fed the low- or medium-protein diet, compared with territorial aggression when fed the high-protein diet. This effect was attributable to a marked reduction in aggression in a subset of the group in which aggression was a result of fear. The conclusions of the research were that a reduction in dietary protein content is not generally useful in the treatment of behavior problems in dogs but may be appropriate in dogs with territorial aggression that is a result of fear.

In 2000, De Napoli et al. evaluated the effect of high and low protein diets with or without TRP supplementation on behavior of dogs with dominance or territorial aggression and hyperactivity.

In each group, 4 diets were fed for 1 week each in random order with a transition period of 3 days between each diet. Two diets had low protein content (approximately 18%), and 2 diets had high protein content (approximately 30%). Two of the diets (1 low-protein and 1 high-protein) were supplemented with TRP (1.45 g/Kg). Owners scored their dog's behavior daily, by use of customized behavioral score sheets. Mean weekly values of 5 behavioral measures and serum

concentrations of 5-HT and TRP were determined at the end of each dietary period. The result showed that for dominance aggression, behavioral scores were highest in dogs fed unsupplemented high-protein rations. TRP-supplemented low protein diets were associated with significantly lower behavioral scores than low-protein diets without TRP supplements. Significant differences were not detected among behavioral groups or diets for plasma TRP or serotonin concentrations Both of the TRP-supplemented diets produced higher TRP:LNAA serum ratio, than the 2 unsupplemented diets; the higher ratio might have caused a greater proportion of TRP crossing the blood-brain barrier, increasing brain serotonin concentration and decreasing aggression. A factor that may have been operating regarding the high-proteins diets is that increased dietary protein concentration increases plasma concentrations of tyrosine and phenylalanine, which are both catecholamine precursors this change could effectively reduce the threshold for aggression (Haller at al., 1998; Stoddard et al., 1986).

In the study of Bosch and colleagues (2009), for 8 weeks, privately owned dogs were fed a control diet or a diet containing 2.6-fold more TRP than the control diet. A third diet fortified with TRP, beet pulp, salmon oil, soy lecithin, and green tea extract was studied for its potential in other dogs. Owners reported on their dogs' behavior in the home-situation by filling out a web-based questionnaire before the onset of dietary treatment and after 4 and 8 weeks of feeding the diets. The dogs were subjected to behavior tests before and after 8 weeks of dietary treatment. The tests included open-field situations and owner-separation procedures and were set up to measure anxiousness. Blood was collected after 8 weeks from dogs in the control and TRP groups for evaluation of plasma amino acid concentrations. Intake of the TRP supplemented diet significantly increased plasma TRP concentrations by 37.4% and its ratio with large neutral amino acids by 31.2% compared to the control diet but owners did not report on behavioral changes that could be attributed to a specific dietary treatment. Also, the dogs' responses in the behavioral tests, including those in saliva cortisol, were unaffected after 8 weeks of consuming the TRP supplemented food. The authors concluded that intake of diets supplemented solely with TRP or in combination with beet pulp, salmon oil, soy lecithin, and green tea extract does not change (anxiety-related) behavior in privately owned dogs that do not show clear signs of abnormal behavior.

In 2012, Leon and colleagues assessed the suitability of different types of blood samples for measuring circulating 5-HT in canine clinical studies and investigated the relationship between the serotonergic system and canine aggression. The mean 5-HT concentration in aggressive dogs was significantly lower than in non aggressive dogs in all the assayed samples. These findings suggest an inverse relationship between the activity of the serotonergic system and canine aggression.

Kato et al. (2012) tested the effectiveness of a diet supplemented with L-tryptophan and alpha-casozepina, a decapeptide derivative of casein hydrolysis alfa-S1-casein, in situations of acute stress such as a visit to the veterinarian. Stress levels were evaluated by assessing the relationship between cortisol and urinary creatinine and questionnaire C-Bark (Serpell & Hsu, 2001). After seven weeks with the diet, the authors witnessed a significant reduction in the scores for the behaviors: aggression toward strangers, fear of strangers, fear not social and sensitivity to physical contact.

In a study of 2013 (Anzola et al., 2013), fifty-three sheltered dogs exhibiting high anxiety levels were studied for a period of six weeks in order to evaluate clinical responses to treatment with TRP. The effect of the diet on the behavior and fecal cortisol levels were assessed weekly throughout the study. The animals were randomly separated into two groups, according to the amounts of administered TRP; the Trp-1 group received 0.6% of TRP and the Trp-2 group received 1.2%. The social behavior of the two groups, during the final week, was significantly different ($P<0.05$). The results indicated that the dogs spent less time engaged in abnormal behaviors ($P<0.05$ and $P<0.001$) than in other behaviors. Likewise, significant differences in cortisol levels ($P<0.05$) were detected between the first and the sixth week. Cortisol values were lower, although not significantly ($P>0.05$) between the fifth and first week of treatment.

In a same year of the Anzola's research, in a study carried out by Cannas et al. (2013), a supple-

ment of Valeriana officinalis, Melissa officinalis and L-tryptophan was given to a group of 15 dogs. The symptoms related to anxiety of each dog were identified through specific questionnaires provided to the owners. After two months a follow-up questionnaire was given to the owners. Dogs, treated with the supplement, obtained significantly lower scores than the control group, showing significant improvements in the frequency and intensity of some of the symptoms analyzed, such as: "follow the owner", "do not answer the call", "aggressive manifestations" "eliminations inappropriate", "excessive vocalizations," "destructions" and "coprophagy" (Cannas et al., 2013).

In 2018, Gazzano and colleagues (Gazzano et al., 2018) conducted a research with the aim to investigate the possibility to augment the serum TRP bioavailability, modifying the diet. In fact, at the current state of knowledge two factors, appear influencing, in a decisive way, the L-tryptophan bioavailability: its relative concentration respect LNAA (large electrically neutral amino acids: tyrosine, phenylalanine, leucine\isoleucine and valine) (Fernstrom & Wurtman, 1972) and its binding to albumin (Chaouloff, 1993). Although TRP is contained in most of the proteins, its real availability depends on the amount of other aminoacids present, and particularly of the amino acids LNAA. Both TRP and LNAA are transported through the blood-brain barrier by the same carrier for which the LNAA have, however, higher affinity. Therefore, in a diet rich in proteins, TRP is available to a lesser degree than the other amino acids. The authors evaluated the plasma ratio between TRP and five large neutral amino acids (isoleucine + leucine + phenylalanine + tyrosine + valine) (5LNAAs) after a single meal with high carbohydrates level. Five female Labrador Retrievers were involved. Each dog was fed three different meals: M1 (a mix of puffed rice, minced meat and olive oil), M2 (puffed rice and olive oil) and M3 (commercial dry food usually consumed) once in the morning for one single day every 30 days. Blood was collected right before the first meal (T0) and after 2, 4, 6, 8, 10 and 24 h. Plasmatic TRP concentrations showed no significant difference between M1, M2 and M3 samples at any sampling time. M2 led to a decrease in 5LNAAs levels and consequently led to a significant higher TRP/5LNAAs ratios in the 6 h period after the provision of carbohydrates, compared to both M1 and M3. In addition, the mean TRP/5LNAAs ratio was significantly higher in M2 than in M3 at t8 and t10. These results indicate that meal composition affects TRP/5LNAAs ratio and possibly, TRP bioavailability.

The same research group (Gazzano et al., 2019) evaluated, in a pilot study, the effects of a carbohydrate-based diet on serotonin blood concentrations in phobic dogs. For this study were recruited three dogs who have received by a veterinary behaviorist a diagnosis of interspecific social phobia. The dogs were fed 2 daily meals (at 8.00 A.M. and 4.00 P.M.), the first meal was exclusively carbohydrate-based (puffed rice) whereas the second one was composed by the commercial diet. Blood was collected every 21 days after 8 hours from carbohydrate meal to determine the levels of 5-HT, TRP and cortisol. Statistical analysis did not reveal any significative difference between the serum concentrations of 5-HT, TRP and cortisol, at the different times, despite a tendency to increase during the time.

Finally, in 2021 a study of Riggio and colleagues (Riggio et al., 2021) aimed at investigating possible differences in dogs' serum TRP and 5-HT concentrations according to their behavioral response to a potentially stressful procedure. Thirty-nine physically healthy shelter dogs, 15 females and 24 males, mean age = 5.6 years, were categorized by a certified veterinary behaviorist according to their behavioral response to medical examination and blood collection, in: relaxation, stress signals, tension without growling, tension with growling, escape attempts, and aggression attempts. The results showed no significant difference in TRP nor 5-HT serum concentrations among different categories of dogs; however, some categories were underrepresented (relaxation = 20.5%, stress signals = 30.8%, tension without growling = 43.6%, tension with growling = 5.1%, escape attempts = 0%, aggression attempts = 0%). No correlation between serum TRP and 5-HT concentrations was found. Serum 5-HT levels do not seem to be associated with dogs' behavioral response to a stressful situation nor with serum TRP concentrations.

Conclusion

As pointed out by Dipace (2015), "based on these studies the use of TRP in the behavioral clinic of the dog is still controversial. While there is evidence of efficacy of products containing TRP and other substances during anxiety syndrome and stress, the use of only TRP in the control of canine aggression has given, until now, inconclusive results. Some authors recommend feeding aggressive dogs with high carbohydrate and low protein diets in order to help TRP to overcome the emato-encephalic barrier". Further studies will be needed to clarify the efficacy of diet modifications on dog behavior.

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Triptofano, serotonina e comportamento del cane

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Sintesi

La serotonina è stata isolata per la prima volta da Rapport e colleghi, dopo decenni di ricerche su una sostanza con attività vasocostrittrice che si sospettava fosse contenuta nelle piastrine.

Dopo la sua scoperta, alcuni studi hanno permesso di identificare la sua localizzazione e funzione in molti tessuti ed organi (cervello, polmoni, reni, piastrine e tratto gastroenterico).

Oltre al suo ruolo nella funzione piastrinica, Brodie & Shore (1957), proposero in primo luogo un ruolo della 5-HT come neurotrasmettitore, basato sulla localizzazione dei recettori 5-HT in aree specifiche del cervello dei vertebrati. Il legame tra metabolismo TRP, serotonina e comportamento nel cane è stato oggetto di notevole interesse e discussione. L'uso del TRP nella clinica comportamentale del cane è ancora controverso. Mentre ci sono prove dell'efficacia di prodotti contenenti TRP e altre sostanze durante la sindrome d'ansia e lo stress, l'uso del solo TRP nel controllo dell'aggressività canina ha dato, fino ad ora, risultati inconcludenti.