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Evolution of Elevated Plus Maze Test (Anxiety) and Porsolt Swimming Test (Depression) Parameters in Wistar Female Rats Treated with Low Dose of Toluene from the 4th to 14th day of Pregnancy: Implication of Progesterone to Protect GABAergic route

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Abstract

Background: The endocrinal and physiological consequences, following the sub chronic administration of toluene (Tol), were examined in pregnant Wistar rats.

Methods: A quarter of lethal dose concentration (LD50) of toluene was injected daily in IP to pregnant rats between the 4th and the 14th day of pregnancy. Virgin female rats were also used as a basis of comparison and obey to the same experimental protocol. Blood samples and recordings of sequences of behavior in elevated plus maze was made in 7th, 14th and 21st day of pregnancy. The measurement of the serum levels of progesterone were carried out at the 7th day of gestation (3rd day of treatment), the 14th day (last day of treatment) and one week after delivery (14th day after discontinuation of therapy). After delivery, which occurs at the 21st day, we tested the effectiveness of a GABAergique agonist, the clonazepam, during the forced swimming test.

Results: Our results showed that the application of toluene neurotoxic stress during 10 days caused endocrine (plasma progesterone) and behavior (anxiety) disruption that appear to be irreversible in virgin female rats treated with toluene. For cons, the same treatment associated with pregnancy revealed a healing effect 14 days after stopping treatment. Inefficiency of both clonazepam and toluene treatment in virgin female rats was observed. This treatment was effective in pregnant rats treated only with toluene.

Conclusion: Pregnancy appears to play a moderating effect on harmfulness and neurotoxicity of toluene, by protecting GABAergic route. Progesterone, as a neuroactif steroid, plays an important role in this protection.

Keywords: Pregnancy; Toluene; Progesterone; Neuroactif steroids; Anxiety; Depression

Introduction

Last thirty years, it has been shown that in female rats, pregnancy causes behavioral changes in response to different environmental stimuli [1]. Galea et al. [2] found an increase in memorable performances in the Morris maze test. A reduction in anxiety was also reported in elevated plus maze [3]. In the forced swimming test, progesterone seems to be antidepressant-like [4]. All these data are in favor of a depressive-like activity of pregnancy and progesterone [5]. Steroid hormones exert their effects not only by binding to intracellular receptors [6] but can also bind to specific neurotransmitter receptors and alter neuronal excitability [7]. The steroid molecules that act as neuromodulators in this mode are called „neuroactive steroids" (NAS) [7]. The NAS involved in mood disorders include progesterone, pregnenolone, pregnenolone sulfate (PS), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and a number of NAS 3α-reduced.

The NAS can be derived from the peripheral (circulating hormones in the blood) or are produced locally in the brain from cholesterol [8]. Corticosteroids and some gonadal steroids (estrogen and progesterone) are also able to cross the blood-brain barrier to act as NAS. Their synthesis occurs in several sites in the nervous system, including neurons, oligodendrocytes, Schwann cells and type 1 astrocytes [8]. These NAS increased during pregnancy and return to pre-pregnancy levels within 6-7 weeks postpartum [8]. The NAS seem to have several biological roles, including neurodevelopment, neuroprotection [9] and are involved in physiological processes such as sleeping and cognitive

function [10]. Mounting evidence suggests that NAS are involved in the pathophysiology of several neuropsychiatric diseases [8], including affective disorders [11,12] and may be particularly involved in memory disorders, sleeping disorders and anxiety, which are commonly associated with depression [12]. From these data, we hypothesized that pregnancy could provide the body with a protective barrier in response to nerve attacks (stress, inhalation of toxic substances, etc.) and this protection would, a priori, occur via production of NAS extensively developed during pregnancy.

The use of organic solvents such as toluene is the best example to test the capabilities of the pregnancy in protecting the body [13]. The exposition of a living organism to toluene, often causes variable physiological responses which can affect basic biological processes (reproduction, development, etc). For example, gestational stress, in laboratory rats, associated with its administration, tend to cause both the mother and its descendant, anxiety disorders and conduct

to drug addiction [14]. The pharmacological and neurotoxic reactions associated with administration of toluene include effects on the central nervous system (CNS): GABAergic, glutamate, serotonin and dopamine systems [15]. In this context and considering the involvement of these products in the behavioral changes that occur during pregnancy, we chose an experimental approach based on the administration to Wistar pregnant rats, low dose (1/4 of the LD50) of a neurotoxic product, the toluene, which is the preferred route of GABAergic neurons (GABA is the only inhibitory neurotransmitter in the central nervous system). To test the alteration of the GABAergic pathway following administration of toluene and the likely role played by pregnancy in protecting this path, we used, seven days after delivery, the forced swimming test (FST), an animal model of depression, which we used as an antidepressant-like GABAergic agonist, clonazepam. Further investigations were conducted, including the elevated plus maze (EPM) and hormone assays (progesterone). This procedure would allow us to confirm if the products of pregnancy (NAS: progesterone) may protect the GABAergic pathways.

Material and Methods

The experimental protocol was approved by the Scientific Committee of our faculty which is consistent with the principles of Animal Health (NIH Publication No. 85-23, revised 1985).

Animals and housing

Adult, intact female Wistar rats (*Rattus rattus*) (n=10) were obtained from Pasteur Institute of Algiers. Rats were group housed in a temperature and humidity controlled room on a reverse light cycle (lights off at 8:00 a.m.) with ad libitium access to water and rat chow in their cages.

Determination of sexual receptivity

Daily (between 10:00 and 11:00 a.m.), females were vaginally masked and paired briefly with a stimulus male (that was conditioned to show consistent, high levels of sexual contact). Sexual receptivity was determined by the response of experimental females to stimulus male investigation. Rats that demonstrated receptive (lordosis) and proceptive behaviors (hopping, darting and ear wiggling) were considered to be in behavioral estrus, while those that exhibited aggressive behaviors (vocalizing, defensive posturing, boxing and avoidance) were not considered in behavioral estrus. Vaginal cytology was used to determine estrous cycle.

After identifying the phases of the rats estrous cycle, we divided them into four experimental groups (n=10):

V group: Virgin female rats received an IP injection of olive oil per day from day 4 to day 14.

TolV group: Virgin female rats received an IP injection of toluene, from day 4 to day 14.

P group: pregnant rats. Male rats were introduced into the cages for mating regardless of their weight at a rate of one male per female. Fertilization is confirmed by the presence of mucus plug in the vaginal smear, which corresponds to the first day of gestation. The rats received an IP injection of olive oil per day from day 4 to day 14.

TolP group: Pregnant rats treated with toluene. Gestation was performed in the same way as for the P group and all pregnant rats receiving 1 IP injection of toluene per day from day 4 to day 14 of gestation.

Toluene administration

The administration of toluene was carried out by IP in a dose of 332 mg/kg of body weight corresponding to ¼ of the lethal dose LD50 determined by IUCLID [16]. The treatment lasted 10 days and was conducted between the 4th and 14th day, due to a daily injection of 2 ml of a solution containing 41.5 g of toluene/l of olive oil. Virgin groups (baseline) and untreated pregnant groups (P) rats received, using the same protocol, an injection of 2 ml of olive oil.

Behavioral testing

Forced swimming test: We hypothesize that pregnancy appears to play a moderating effect on harmfulness and neurotoxicity of toluene, by protecting GABAergic route. To test this hypothesis we used the test of Porsolt or forced swimming test. In rats, some behavioral changes occurring may be analyzed in the forced swimming task (FST), which is designed to test the antidepressant profile of drugs. The present study was aimed to analyze in pregnant rats, after delivery, the effectiveness of an agonist GABAergic (clonazepam) those behavioral changes displayed in the FST [17]. This approach can we confirm whether the GABAergic pathway is impaired in four experimental groups. Rats were placed in an aquarium of 21° to 22°C water filled to a depth of 35 cm for a 15-min pretest. Injections (saline or clonazepam) were given 23.5, 5 and 1 hr before a 5 min test swim. The water depth of 35 cm allowed the rats to swim or float. Clonazepam was administered subcutaneously in a volume equivalent to 2 ml/kg at a dose of 0.25 mg/ml [18]. Saline (0.9%) was also administered subcutaneously in a volume equivalent to 2 ml/kg. The swimming session on each day was videotaped for behavioral analysis. The time of immobility, swimming and climbing are calculated.

Elevated plus maze test: Behavior in the elevated plus maze is also utilized to assess anxiety behavior [19]. We measured two behavioral variables: the number of entries into open arms and the amount of time spent in open arms. Anxious animals are expected to make fewer entries into open arms and to spend less time in open arms than are non-anxious animals. The plus maze was elevated 50 cm off the ground and consisted of four arms (49 cm long and 10 cm wide). Two arms were enclosed by walls 30 cm high and the other two arms were exposed. As per previous methods, rats were placed at the juncture of the open and closed arms and the number of entries into and the amount of time spent on, the open and closed arms were recorded during a 10 min test. Time spent on the open arms is an index of anxiety and the total number of arm entries is measure of motor activity.

Sample preparation and ELISA assay of LH and progesterone

The blood sample from retro-orbital route is collected on the 4th, 14th and 28th day (baseline and Tol) and after delivery (post partum: 28th day) (P_{28} and $PTol_{28}$). Blood samples were centrifuged at 5000r/min to be used for hormone assays of progesterone. Progesterone levels were measured by the conventional ELISA test kit BIOTECH (reference: 7/306 for progesterone). The measurement is made using a TECAN ELISA reader equipped with Magellan computer software that automatically calculates the standard range and gives us directly the value of the hormone to the desired unit.

Statistical analysis of results

Data are presented as mean ± SEM. Data were analyzed by one-way ANOVA and Newman and Keuls as the post hoc test, while the results from binding assays were analyzed by the Student's t test. Results were considered significant at $p < 0.05$.

Results

Change in progesterone levels (ng / ml)

According to Figure 1, changes in progesterone appear to be more sensitive to both the processing pregnancy to toluene. Pregnancy causes an increase in progesterone levels on day 7 (V_7: 1.09 ± 0.17 vs P_7: 1.671 ± 0.12). This increase is greater at day 14 of pregnancy (P_{14}:

3.216 ± 0.07 vs P_7: 1.671 ± 0.12). Seven days after delivery, the level decrease to a value comparable to virgin (V_{28}: 0.932 ± 0.022 vs P_{28}: 1.271 ± 0.18). At day 7, treatment with toluene in virgin groups led to a very highly significant increase of progesterone levels compared to virgin animals ($TolV_7$: 1.57 ± 0.16 vs V_7: 1.09 ± 0.17). Than it increase at day 14 ($TolV_{14}$: 1.488 ± 0.091 vs V_{14}: 1.17 ± 0.094) to achieve a very low value 14 days after stopping treatment ($TolV_{28}$: 0.684 ± 0.094 vs V_{28}:
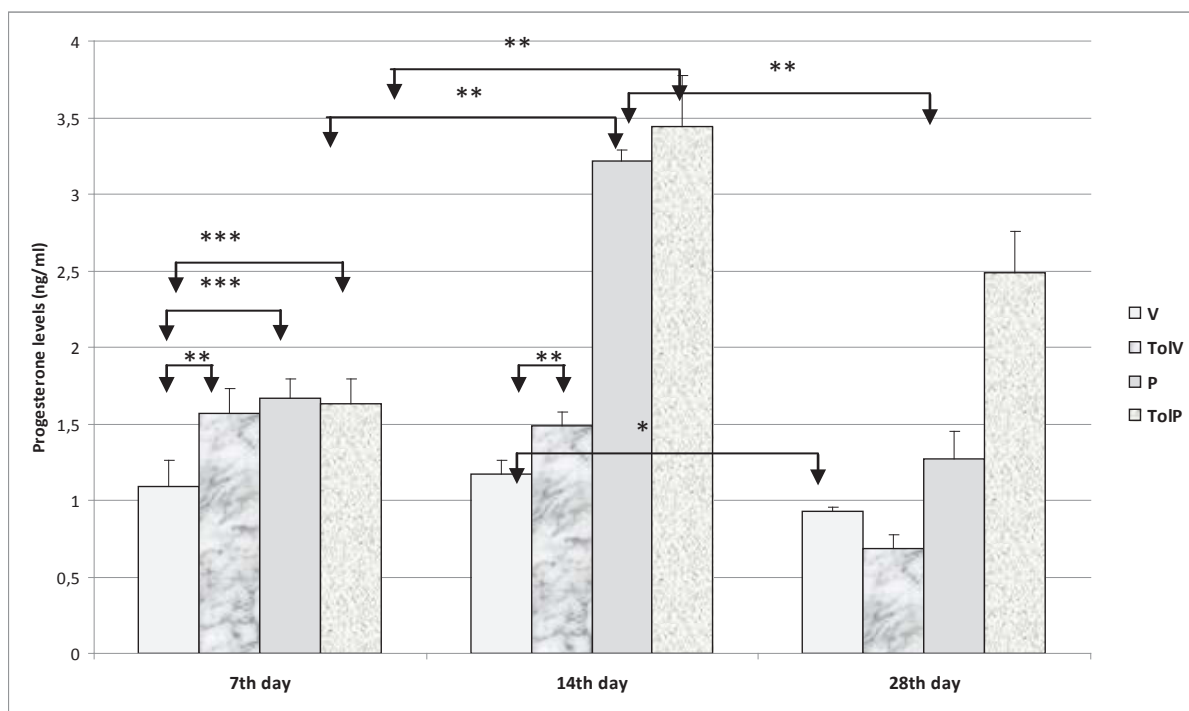

Figure 1: Change in plasma progesterone levels (ng / ml) following treatment with toluene in Wistar rats females (n=10 ; * p < 0,05, ** p < 0,01, *** p < 0,001).

Behavior/groups	Rats	7th day	14th day	28th day
Time spent in center (Sec)	V	35,377 ± 10,23	11,25 ± 6,701	13,1 ± 2,33
	TolV	5,5 ± 2.30	2,5 ± 0,707	8,88 ± 2,64
	P	45,5 ± 3,535	3,535 ± 1,339	3,85 ± 0,678
	TolP	54 ± 12,055	16,25 ± 11,982	24,16 ± 1,732
Time spent in open arms (Sec)	V	50,25 ± 3,5	39,75 ± 3,818	0,00 ± 0,00 b**
	TolV	47 ± 36,76	0,00 ± 0,00 b**	5,38 ± 1,61 b***
	P	127 ± 10,89 a***	144,95 ± 10,5 a***	3,69 ± 0,739 b***
	TolP	128,75 ± 6,88	101,5 ± 9,47	16,58 ± 1,859
Time spent in closed arms (Sec)	V	249 ± 41,705	230,75 ± 74,428	286,9 ± 6,33 b**
	TolV	253 ± 25,3	293,5 ± 9,192	285,74 ± 17,25 b**
	P	195,5 ± 80,672	135 ± 148,492	288,67 ± 13,47b**
	TolP	125,116 ± 82	175 ± 2,403	262,50 ± 24,56 b*
number of entries in open arms	V	3,52 ± 2,449	2,5 ± 1,732	0,00 ± 0,00 *
	TolV	4 ± 1,414	0,00 ± 0,00 b**	0,5 ± 0,070
	P	7,071 ± 1,052 a**	6,414 ± 0,10 a**	0,75 ± 0,095 b***
	TolP	6,5 ± 0,707	2 ± 1,825	1,25 ± 0,125
number of entries in closed arms	V	3,5 ± 1,914	3,75 ± 1,2	1,41 ± 0,021
	TolV	1,5 ± 0,701	1 ± 0	1,5 ± 0,070
	P	9,5 ± 0,707	1 ± 0	1 ± 0
	TolP	2,5 ± 1,732	4,75 ± 1,778	6,5 ± 1,91

Table 1: Changes in the parameters of the elevated plus maze following treatment with toluene in female Wistar rats
a : V vs TolV
b : 7th day P vs 14th day P vs 28th day
(n=10 ; * p < 0,05, ** p < 0,01, *** p < 0,001)

0.932 ± 0.022). In pregnant rats, progesterone levels increase at day 7 of pregnancy (P_7: 1.671 ± 0.12; $TolP_7$: 1.632 ± 0.162 vs V_7: 1.09 ± 0.17). This increase is greater at day 14 of pregnancy (P_{14}: 3.216 ± 0.07 vs P_7: 1.671 ± 0.12; $TolP_{14}$: 3.444 ± 0.33 vs $TolP_7$: 1.632 ± 0.162). After delivery, the progesterone levels come down only in pregnant rats not treated with toluene (P_{28}: 1.271 ± 0.18 vs P_7: 1.671 ± 0.12). However, in toluene treated, progesterone levels remain relatively increased ($TolP_{28}$: 2.49 ± 0.27 vs $TolP_7$: 1.632 ± 0.162). Furthermore, it should be noted that treatment with toluene only led to a highly significant increase in progesterone (V_7: 1.09 ± 0.17 vs $TolV_7$: 1.57 ± 0.16) and the association of gestation to treatment attenuated the effect of toluene (P_7: 1.671 ± 0.12 vs $TolP_7$: 1.632 ± 0.162; V_{14}: 1.17 ± 0.094 vs $TolV_{14}$: 1.488 ± 0.091; P_{14}: 3.216 ± 0.07 vs $TolP_{14}$: 3.444 ± 0,33).

Elevated plus maze test

Table 1 shows the changes of parameters of the elevated plus maze. We found that from a session (session 1 and 2) to another (session 3), animal behavior has changed, because it flows less freely in the open arms (time and number of entries in these arms) [Time spent in open arms (Sec) : V_7 : 50,25 vs. V_{28} : 0,00(p < 0.01) ; V_{14} : 39,75 vs V_{28} : 0,00 (p <0.01)] ; [Number of entries in open arms: V_7: 3,52 vs. V_{28} : 0,00 (p < 0.01); V_{14} : 2,5 vs. V_{28} : 0,00(p<0.01)]. The rats are more anxious during the third session (28th day). If we refer to Figure 1, where we found a drop in progesterone levels during the third session (28th day), we can conclude that these measures clearly confirm that progesterone may exert an anxiolytic effect which is strongly more important during sessions 1 (7th day) and 2 (14th day). In pregnant rats we have seen similar fluctuations in the previous case (virgin), with a decrease of the anxiolytic parameters after delivery [Time spent in open arms (Sec): P_7: 127 vs. P_{28}: 3, 69 (p<0.001); P_{14}: 144,956 vs. P_{28}: 3, 69 (p < 0.001)]; [Number of entries in open arms: P_7: 7,071 vs. P_{28}: 0, 75(p<0.001); P_{14}: 6,414 vs. P_{28}: 0, 75(p < 0.001)]. Furthermore, by comparing the pregnant group to virgin group, we can see clearly that pregnancy greatly reduces anxiety, thereby increasing anxiolytic parameters: [Time spent in open arms (Sec) : P_7 : 127 vs. V_7 : 50,25 (p < 0.001) ; P_{14} : 144,956 vs. V_{14} : 39,75 (p < 0.001)] ; [Number of entries in open arms: P_7: 7,071vs. V_7: 3,52 (p < 0.01); P_{14} : 6,414 vs. V_{14} : 2,5(p < 0.01)]. Referring to Figure 1, where we noted an increase in progesterone levels in pregnant rats on day 7 and day 14, we confirm once again that progesterone exerts an anxiolytic effect during sessions 1 (7th day) and 2 (14th day). Treatment with toluene for 3 days (the 7th day of sampling) did not cause any change in level of anxiety, where the time spent in open arms and the number of entries in these arms were not altered for the virgin group [Time spent in open arms (Sec): Tol_7: 47 vs. V_7: 50, 25 (NS)]; [Number of entries in open arms: Tol_7: 4 vs. V_7: 3, 52 (NS)]. The 10th day of treatment (the 14th day of sampling), anxiety has increased dramatically in this group (TolV) compared to the virgin group (V), resulting in a number of entries in open arms virtually zero [Time spent in open arms (Sec): Tol14: 00 vs. V14: 39, 75 (p<0.01)]; [Number of entries in open arms: Tol14: 00 vs. V14: 2, 5(p < 0.01)]. At day 28, we obtained the opposite case where the rats treated with toluene are less anxious than the virgin. It is as if these animals (treated with toluene) have more difficulty coping. This adjustment difficulties, has not been found in pregnant animals treated with toluene which we obtained a profile of behavior comparable to that of untreated pregnant rats. This observation gives us the impression that pregnancy facilitates the adaptation of rats to treatment. In addition, we found that anxiety increases significantly 7 days after delivery (Table 1). This increase is related to the significant drop in progesterone (Figure 1).

Behavior	Swimming (sec)	Immobility (sec)	Climbing (sec)
V without Clonazepam	32,45 ± 12,69	249,56 ± 32,14	47,865 ± 12,57
V_{28} + Clonazepam	98,08 ± 29,73	127,74 ± 52,6	74,17 ± 22,94
	***	**	***
P_{28} + Clonazepam	127,2 ± 0,63	119,24 ± 2,32	53,56 ± 2,52
	***	***	
$TolV_{28}$ + Clonazepam	42,18 ± 11,67	257,46 ± 21,57	20,35 ± 6,92
			*
$TolP_{28}$ + Clonazepam	77,14 ± 29,09	155,06 ± 30,77	67,82 ± 15,88
		**	

Table 2 : Effect of Clonazepam (GABA agonist) on the parameters of forced swimming in pregnant and virgin Wistar rats (P and V) and treated with toluene (TolV, TolP)

(n=10 ; * p < 0,05, ** p < 0,01, *** p < 0,001).

Forced swimming test

In the forced swimming test, we found that clonazepam is effective in Virgin group (Table 2), resulting in a significant reduction in the immobility time (V without Clonazepam: 249, 56 vs. V_{28} + Clonazepam: 127, 74). Clonazepam is also effective in all other groups (V without Clonazepam : 249,56 vs. P + Clonazepam : 119,24 ; $TolP_{28}$ + Clonazepam : 155,06) except TolV (Table 2), where the immobility time did not change significantly (V without Clonazepam : 249,56 ± 32,14 vs. $TolV_{28}$ + Clonazepam : 257,46 ± 21,57).

Discussion

In our study, measurements of progesterone in pregnant rats (Figure 1) showed a very significant increase on day 7 and day 14 of pregnancy. This increase is accompanied by a decrease in anxiety in elevated plus maze. This suggests that progesterone appears to exert an anxiolytic effect. Indeed, very recently, it was shown that inhibition of progesterone increases immobility in the FST in female mice and blocking progesterone receptors, time becomes less important [20]. Ovariectomized mice show decreased immobility during the FST [20] and tail suspension test (TST) [4] immediately after treatment with progesterone. Some authors have suggested that the antidepressant-like effects resulting from administration of progesterone is the result of the conversion of progesterone to other NAS such as allopregnenalone [21]. The increase in progesterone levels in plasma and brain is accompanied by an increase of allopregnenalone [22]. The majority of allopregnenalone found in the brain is synthesized in the periphery [22]. Progesterone is converted into dihydroprogesterone (DHP) and then allopregnenalone, respectively, by the enzyme 5α-reductase and 3α-hydroxy steroidedeshydrogénase (3α-HSD). Alternatively, progesterone receptor modulates neurotransmitters that might be the cause of their antidepressant effects, for example by increasing the activity of GABAA receptors [23]. In a recent study by Bridges and Starkey [20], progesterone administered intraperitoneally to gerbils, decreased anxiety observed in the dark-light box test. Some research suggests that brain-derived neurotropic factor (BDNF) is decreased in depressed humans [24] and in animal models of depression [25] and that the increase in BDNF is necessary to exercise the effect of an antidepressant [25]. BDNF influences the activity of the hypothalamic-pituitary-adrenal (HPA) [26]. Naert et al. [25] found that NAS activity enhances the HPA axis. The Trilostane is an inhibitor of 3-β-hydroxysteroid dehydrogenase, the enzyme that converts pregnenalone to progesterone [27]. When administered to mice, it increases levels of a number of NAS, reduces immobility in the FST and increases levels of BDNF in the hippocampus [27]. Major depression in men was associated with high levels of circulating adrenal

corticosteroids [26]. Treatment with exogenous allopregnenalone can have an antidepressant effect, reducing the time of immobility during the FST in mice [27] and ovariectomized rats [28]. The allopregnenalone also increases levels of BDNF in the hippocampus, hypothalamus and amygdala [28]. Inhibition of 5α-reductase, an essential enzyme for the conversion of progesterone to allopregnenalone causes the increase of immobility in the FST in rats [29]. The allopregnenalone is also able to potentiate the inhibition of $GABA_A$ receptors, mediated by serotonergic neurons in the dorsal raphe neurons, which may have an impact on mood [30].

We noted that treatment with toluene for 3 days (the 7th day of sampling) did not cause any change in level of anxiety and the 10th day of treatment (the 14th day of sampling), anxiety has increased dramatically in this group (TolV) compared to the virgin group (V). At day 28, we obtained the opposite case where the rats treated with toluene are less anxious than the virgin. We concluded that these animals (treated with toluene) have more difficulty coping. The process of adaptation of animals to different types of stress (psychological, physical, chemical, etc.) involves neuroendocrine mechanisms in which the HPA axis plays a key role [26]. At the end of pregnancy, the reactivity of the HPA axis to stress is considerably reduced, which is reflected by lower secretion of ACTH (by the anterior pituitary) and corticosteroids (adrenal cortex) and a low expression of mRNA encoding CRH in the nucleus para ventricular hypothalamus (PVN). This decrease in reactivity is considered as a form of adaptation to stress. It aims, firstly, is to reduce fetal exposure to maternal glucocorticoïds to minimize the maximum risk of embryo mortality and birth and also the adaptation of the mother for a successful pregnancy [22,31]. It was reported that estradiol and progesterone are not directly involved in the suppression of stress-related HPA activity, while the metabolites mentioned above, such as allopregnenalone, play a crucial role [31]. During the late phase of pregnancy, production of NAS is increased in the brain while the activity of 5α-reductase is important in the hypothalamus [32]. The mRNA expression of 5α-reductase increases in the nucleus solitary tract (NTS) and that of the 3α-HSD in the paraventricular nucleus (PVN) [32]. The expression of these enzymes during the late phase of pregnancy is not well documented. According to Brunton PJ & Russell JA increased prolactin levels intervene in the regulation of 5α-reductase. During pregnancy, high levels of allopregnenalone diminish the response of the HPA axis to stress. This is confirmed in the study of Brunton & Russell [32] where the administration of an inhibitor of 5α-reductase, finasteride, causes an activation of the HPA axis. In the same study, it was shown that administration of allopregnenalone in virgin female rats also attenuates the stress-related HPA activity. Treatment of rats with estrogen alone and a combination of estrogen and progesterone has been ineffective in the suppression of the activity of the HPA axis stress-related [32]. The same result was obtained with progesterone alone and dihydroprogesterone [32]. This is found in our results, where the fluctuations of progesterone (Figure 1) in animals treated with toluene, outside the context of pregnancy, indicate a loss of adaptive capacity compared to pregnant animals treated with toluene. The levels of anxiety recorded in the two pregnant groups (Table 1) could also be explained by this factor. The allopregnenalone acts by modulating the activity of $GABA_A$ receptors. This effect is confirmed in our results (Table 2) where the administration of a GABAergic agonist, clonazepam, was ineffective in virgin rats treated with toluene and effective in all other groups. It seems that the GABAergic neurons and/ or their receptors were altered, following to administration of toluene in virgin rats. Indeed, it was shown that toluene causes important neurotoxic reactions in the central nervous system: GABAergic,

glutamatergic, serotonergic and dopaminergic systems [13-15]. In pregnant rats, the allopregnenalone protects neurons against potential toxicity by acting on the GABAA receptor chloride channels by keeping them open longer [33] and, as noted above, by increasing the levels of BDNF in the hippocampus, hypothalamus and amygdala [24,25]. The inhibitory activity of allopregnenalone held on CRH neurons in the PVN [33]. The increase in active allopregnenalone GABA-ergic neurons will cause a decrease in the expression of CRH neurons in the PVN and thus, a decrease in HPA activity. This anxiolytic effect was observed in our results (Table 1).

Conclusion

In our study, treatment with toluene in virgin rats caused various problems, both physiological and behavioral. In pregnant rats, the same treatment did not cause the same disorders. Indeed, the physiological and behavioral indicators show some recovery during the gestation. The strength of our results was on the ineffectiveness of the antidepressant clonazepam, a GABAergic agonist, in virgin females treated with toluene, in the forced swimming, when he was effective, in pregnant rats undergoing the same treatment, where it caused a decrease in the immobility time. In this work, we have shown that the neurotoxicity of toluene preferred route for the GABAergic neurons whose alteration causes anxiety, is the case of virgin rats treated with toluene. In their counterparts pregnant, we reported a paradoxical situation, characterized by the disappearance of anxiety; along with the GABAergic agonist (clonazepam) has been effective, which implies a protection of GABAergic neurons. We explained the neuroprotection by the role played by the allopregnenalone (derived from progesterone) whose secretion during pregnancy is increased. Neuroactive steroid acts by modulating the $GABA_A$ receptor activity specifically on chloride channels of neurons, keeping them open longer and thus, suppress the stress reactivity of the HPA axis. Upon completion of this work, it would be desirable to further investigations, especially in the descendants, to support impact of prenatal exposure on the future development of children.

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