Neurophysiology, Vol. 43, No. 6, March, 2012 (Original Vol. 43, No. 6, November-December, 2011)

Cytokines and Absence Seizures in a Genetic Rat Model

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Neirofiziologiya/Neurophysiology, Vol. 43, No. 6, pp. 554-563, November-December, 2011.

Received July 27, 2011.

We investigated the role of two cytokines, IL-1β and TNF-α, in the development of absence seizures using a genetic model of absence epilepsy in WAG/Rij rats. We administered these cytokines to animals systemically and measured the number of spike-wave discharges (SWDs) in the EEG. We also coadministered IL-1β with the GABA reuptake inhibitor tiagabine and measured the levels of IL-1β and TNF-α in the brain and blood plasma of 2-, 4-, and 6-month-old WAG/Rij rats and animals that served as a non-epileptic control (ACI). We found that IL-1β induced a significant increase in SWDs 2-5 h after administration, while TNF-α enhanced SWDs much later. Both cytokines enhanced passive behavior; body temperature was elevated only after TNF-α. The action of tiagabine was potentiated by earlier IL-1β injection, even when IL-1β was no longer active. Young WAG/Rij rats showed higher levels of TNF-α in blood serum than young ACI rats; the effects in the brain tended to be opposite. The marked differences in timing of the increase in SWDs suggest different time scales for the action of both cytokines tested. It is proposed that the results found after TNF-α are due to the *de novo* synthesis of IL-1β. TNF-α may possess neuroprotective effects. IL-1β might increase GABA-ergic neurotransmission. The changes in the efficacy of antiepileptic drugs related to changes in the cytokine systems may have some clinical relevance.

Keywords: IL-1β, TNF-α, WAG/Rij rats, absence epilepsy, tiagabine, GABA.

INTRODUCTION

The actions of proinflammatory cytokines in the CNS have only begun to be discovered. It is known that their central actions include effects on the hypothalamo-pituitary-adrenal (HPA) axis. Pyrogenic and somnogenic effects of cytokines, as well as modifications of the peripheral immune response, have also been described [1]. Some cytokines have been recently shown to affect neurotransmitters, including monoamines, glutamate, GABA, and acetylcholine (ACh), to modulate the preservation of the synaptic efficacy at excitatory synapses, and/or to affect the expression of various neuropeptides and neurotrophic factors in several brain regions [2-4]. Changes in the neurotransmitter concentrations, some secondary messenger systems, and calcium currents may be consequent to alterations in the amounts of inflammatory cytokines or other inflammatory mediators in parenchymal brain cells, such as glia and endothelium, and may change the excitability of the CNS and alter the susceptibility of exogenously induced or genetically determined types of epilepsy [5-7].

There is also evidence for the relation between the immune system and epilepsy. A recent overview on the role of IL-1β in mainly convulsive types of epilepsy was presented by Sayyah et al. [8] and Vezzani et al. [9, 10]. The role of TNF-α in convulsive epilepsies has been well documented [11-16]. Cytokines, including IL-1β and TNF-α, are more readily expressed following seizures, and seizures can be aggravated when cytokines are added exogenously. Actually, cytokines affect all aspects of seizure pathology, i.e., seizures *per se*, neuronal death, neuronal birth, reactive gliosis, and mossy fiber sprouting [9, 10, 14, 17, 18].

At present, little is known about the role of these cytokines in other, less malign, types of epilepsy, such as childhood absence epilepsy. The availability of well-accepted genetic absence models, such as GAERS (Genetic Absence Epileptic Rats from Strasbourg) and WAG/Rij rats, facilitates the research towards the interplay between seizure activity and cytokines. Rats of the WAG/Rij strain are endowed

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with a genetically determined type of epilepsy [19, 20]. At the age of 2 to 3 months, quite few rats show spike-wave discharges (SWDs) in their EEG, while all 6-month-old rats demonstrate SWDs concomitant with behavioral signs, such as twitching of the vibrissae and accelerated breathing combined with an otherwise immobile posture [21, 22]. Age-matched ACI rats have much less SWDs than WAG/Rij rats [23, 24] and are, therefore, often used as controls.

The role of proinflammatory cytokines in generation of SWDs was recently investigated in WAG/Rij rats using administration of various doses of lipopolysaccarides (LPSs), and an increase in SWDs was found [6]. However, LPSs induce and release numerous proinflammatory mediators, e.g., IL-1β, TNF-α, and IL-6. In this study, we tried to establish separately the role of IL-1β and TNF-α in generation of seizure activity. We have chosen these cytokines since they represent two classes of cytokines expressed in the CNS and are most widely studied in relation to epilepsy [15, 25].

In the first experimental series, we investigated whether these cytokines, when added exogenously, exert an effect on SWDs. Little, if any, is known about the duration of the corresponding effects after peripheral administration. What is known is that a diurnal rhythm exists in expression of effects of IL-1β and TNF-α, and that various cytokines may trigger a cascade of events also affecting neuronal, endocrinological, and behavioral parameters (including the production of cytokines themselves) [26, 27]. Therefore, the expression of SWDs following systemic application of the mentioned cytokines was investigated for 72 h after their administration. Also, the behavior and body temperature were regularly measured considering that IL-1β and TNF-α might facilitate passive behavior, including hypnogenic effects [28].

A question of clinical relevance (whether the efficacy of antiepileptic drugs is altered under the influence of cytokines) was investigated in the second experimental series. We used the antiepileptic GABA-reuptake blocker tiagabine for this purpose considering the well-known and specific mode of action and working mechanism of this agent. Tiagabine, just as all GABA mimetics, is known to increase the intensity of SWDs [29, 30].

In the third experimental series, we established whether the above two cytokines might play a role in the development of absence seizures, by measuring the levels of these agents in the brain and plasma at ages of experimental animals of 2, 4, and 6 months. Two-month-old WAG/Rij rats have no seizures, while all WAG/Rij rats show hundreds of SWDs per 24 h at 6 months [21, 30, 31].

METHODS

The EEG studies in experimental series I and II were carried out on 32 adult (9 to 16 months old) male WAG/Rij rats weighing 266 to 406 g. The EEG study as a part of series III was carried out on male ACI and WAG/Rij rats (young ACI, $n = 6$, young WAG/Rij, $n = 6$, old ACI, $n = 6$, and old WAG/Rij, $n = 7$). Rats were kept under standard laboratory conditions, housed in pairs before surgery, with food and water *ad libitum* available. The room temperature was kept at 22-24°C, and a 12/12 h LD schedule (light from 7.00 to 19.00) was maintained. After surgery, rats were individually housed in Plexiglas cages (36×25×15 cm) suited for EEG recordings. Procedures involving animals and their care were conducted according to the University guidelines that comply with international laws and policies concerning the use of laboratory animals in experimental trials (European Community Council Directive 86/609, OJ L 358, I, December 12, 1987). A local Ethical Committee (RU-DEC) approved the protocol.

Surgical Preparation. Three standard EEG electrodes (Plastic One, MS303/2) were stereotaxically implanted under anesthesia with isoflurane (4.5% for induction and 2-2.5% for maintenance) in oxygen. Electrode coordinates were AP +2.0, L 2.0 and AP –6.0, L 4.0, with a reference electrode positioned on the cerebellum. The stereotaxic atlas of Paxinos and Watson was used as reference for the coordinates. The EEG-electrode set was fixed with acrylic plastic and two screws, which were placed bilaterally over the cortex. The experiments were performed at least 10 days after surgery.

Experimental Protocol. Rats were familiarized for 24 h with EEG recording leads connected to a swivel. Signals were amplified and filtered (limits 100 and 1 Hz).

In experimental series I, independent groups of rats (group size $n = 9$ to 11) were injected i.p. at day 1 (D1) with Interleukin-1β (human, recombinant, 2 μg/kg in 2 ml, Sigma, USA), TNF-α (human, recombinant, 2 μg/kg in 2 ml, Sigma, USA), or a bovine serum albumin (BSA) salt solution (0.5% BSA in phosphate buffer solution, PBS; 2 μg/kg in 2 ml) as the control. The EEG registrations started immediately after administration of the cytokines or

solvent at 13.00 and lasted 72 h. The behavior of the rats was observed everyday through a window from an adjacent room between 13.30 h till 15.30 h for half an hour per rat. We recorded passive behavior (sitting, lying, or standing motionless; only an occasional movement of the vibrissae was allowed) *vs* active behavior (walking, rearing, grooming, digging, eating, and drinking behavioral episodes), as described earlier [32]. The data were off-line analyzed using Observer software [33]. The body temperature was measured manually with a rectal probe in the evening (17.00) and in the morning (9.00 h).

Experimental Series II. This experiment was carried out at least 2 weeks after experimental series I; 14 randomly chosen rats from the first experiment were used. Rats were injected i.p. with BSA or IL-1β (2 μg/kg in 2 ml) at 10 a.m. followed by a tiagabine (3 mg/kg) injection 5 h later (group size $n = 8$ and $n = 6$ for Il-1β and BSA, respectively). The dose of tiagabine (3 mg/kg) was the same as that in our previous studies [29]. The EEG was recorded for 8 h (5 h after the first injection and 3 h or more after tiagabine injection).

In experimental series III, naive rats ($n = 23$) equipped with cortical EEG electrodes (all surgical procedures and recording conditions were identical to those described above) were familiarized for 24 h to EEG leads in the recording cage; the next day, the EEG was recorded for 2 h.

ELISA. Seventy-four rats (ACI and WAG/Rij) were used in the ELISA study. Fifty-one animals were experimentally naive, while 23 ones were used in the EEG part of experimental series III. Rats were 2, 4, and 6 months old. All rats were killed by decapitation, and their brains were rapidly removed at 4°C and frozen on dry ice. The brain tissue was weighed and homogenized in ice-cold PBS (5 g/ml) using a Potter homogenizer (1,000 min^{-1}, 10 strokes). The homogenates were centrifuged for 10 min (5,000 min^{-1}, 4°C). One hundred microliters of the supernatant were taken in duplicate to measure TNF-α and IL-1β contents. The amounts of TNF-α and IL-1β were determined using selective antibodies (Biotrak system, from Amersham Pharmacia Biotech, USA). The absorbance was read at 405 nm. The detection limit was 4.0 pg/ml. The obtained data were expressed in picograms per milligram of the wet brain tissue. For each estimation, 0.416 mg of tissue was used in 50 μl of the investigated solution for IL-1β and in 5 μl for TNF-α.

Analysis of EEG. The EEG recordings were analyzed with a custom-made program that was previously validated. The SWDs were initially identified by this program and subsequently checked by a trained EEG analyst. Criteria for the detection of SWDs can be found elsewhere [22]. The numbers of SWDs per hour are shown below.

Statistical Analysis of the Data. The effects of the exogenous administration of cytokines on SWDs were analyzed with a repeated-measure ANOVA with cytokine (3 levels) as a between-subject factor, and time as a within-subject one. If necessary, univariate analysis was followed by Duncan's post-hoc test ($P < 0.05$). Considering a large number of the variables (time points, hours) in relation to the number of animals, it was not possible to analyze all the EEG data in one large ANOVA. The effects of cytokines might most likely occur in the first few hours after administration; therefore, an initial period (1 to 6 h) after injection (dark period) and the remainder of the day were analyzed. The remainder of the recording period was subdivided into days with blocks lasting 2 h.

The duration of passive behavior and body temperature were analyzed with ANOVA with days as a within-group factor and treatment as a between-group one. The efficacy of the antiepileptic drug tiagabine was statistically evaluated with ANOVA and followed by paired and unpaired Student's *t*-tests.

All outcomes of the ELISA method were analyzed with a single MANOVA with strain (2 levels), age (3 levels), and operation (2 levels) as between-subject variables. Since there were neither main effects of operation nor interactions with operations, the data were pooled in a final two-way MANOVA followed by univariate ANOVA and post-hoc tests.

The number of SWDs, as measured in the comparative study between 2- and 6-month-old WAG/Rij and ACI rats, were analyzed with ANOVA with strain and age as between-group factors. Statistical analysis was done with SPSS, version 10.0, for Windows. Graphics were made with Prism 3.0.

RESULTS

Experimental Series I. The number of SWDs, as recorded over the 72-h period in the control group, demonstrated a circadian pattern with a peak in the number of these complexes within the dark period and a smaller number within the light phase of the 24-h (12/12) light-dark cycle. Significant time-related effects ($F = 5.69$, $df = 11,18$, $P < 0.001$ and $F = 2.51$, $df = 11,23$, $P < 0.05$, respectively) were found on D2

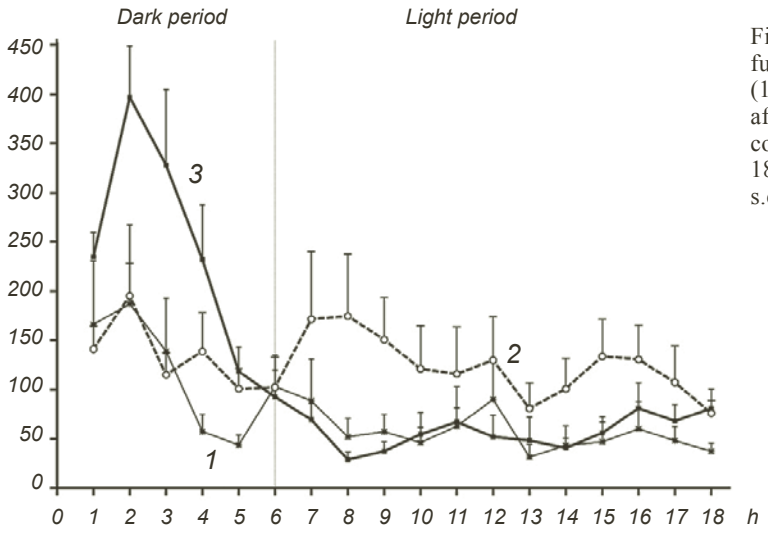

Fig. 1. Total duration of spike-wave discharges per hour as a function of time (h) after injections of bovine serum albumin (1), TNF-α (2), and IL-1β (3). Note the immediate increase after IL-1β injection and the delayed increase in the TNF-α content within the subsequent light period from the 7th to 18th h after injection. In this and other figures, means and s.e.m. are shown.

and D3 (data not shown). On both days, the data could be described with a linear and cubic trend, suggesting that the changes over time were not due to chance and showed a maximum and a minimum, reflecting the circadian distribution of SWDs.

The outcomes of the first 17 h of the EEG study are presented in Fig. 1. The repeated measures ANOVA for the first 6 h after injection showed a time effect ($F = 2.73$, $df = 5,34$, $P < 0.05$), a treatment effect ($F = 7.47$, $df = 2,38$, $P < 0.01$), and a time × treatment effect ($F = 1.99$, $df = 10,70$, $P < 0.05$). The interaction prompted us to analyze the treatment effects per hour with a univariate analysis followed by post-hoc tests. These results are presented in Table 1.

The post-hoc tests revealed that IL-1β enhanced SWD expression during 1 to 5 h after its administration.

The ANOVA for the first light period (7 to 18 h post injection) showed a treatment effect ($F = 5.37$, $df = 2, 37$, $P < 0.01$). The subsequent post-hoc test showed that the number of SWDs was higher for the TNF-α group than that for the Il-1b and control groups. There were no differences between the three groups at D2 and D3.

Passive Behavior. The ANOVA on passive behavior revealed a day-effect ($F = 11.76$, $df = 2,42$, $P < 0.001$) and a significant day × treatment effect ($F = 3.20$, $df = 2,42$, $P < 0.05$). Post-hoc tests showed that passive behavior was more expressed during D1 compared to D2 and D3. The univariate analysis for the data on D1 showed a treatment effect ($F = 4.58$, $df = 2,43$, $P < 0.05$); the post-hoc tests according to Duncan showed that both experimental groups demonstrated more passive behavior than the control group did (Fig. 2). There were no differences between the three groups on D 2 and D 3.

Temperature. The body temperature data are presented in Fig. 3. The data in this figure and the ANOVA's outcomes demonstrate that body temperature was higher in the evening than in the morning ($F = 28.45$, $df = 2,42$, $P < 0.0001$), and that the temperature was higher on D1 compared to that on D2 and D3 ($F = 3.29$, $df = 2,42$, $P < 0.05$, and post-hoc tests). The significant outcomes of the first-order ($F = 2.78$, $df = 2,42$) and second-order interaction ($F = 3.04$, $df = 2,40$) suggest that the temperature was elevated on D1 but only in the evening (first-order)

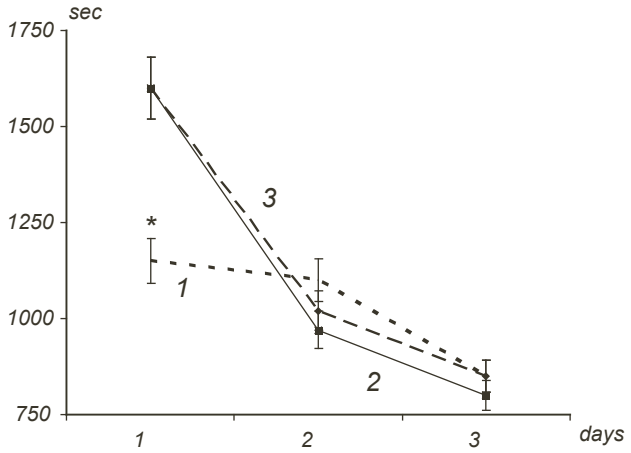

Fig. 2. Duration of passive behavior (sec) after injections of BSA (1), TNF-α (2), and IL-β (3). Noticeable is the high amount of passive behavior 1-3 h after injection of both TNF-α (2) and IL-1β (3), 24 and 36 h; later on, there were no longer behavioral effects of the cytokines. *$P < 0.05$. Other designations are the same as in Fig. 1.

TABLE 1. Number of SWDs and Their Mean Duration (sec) per Hour in WAG/Rij and ACI Rats

Age, months	WAG/Rij rats		ACI rats	
	number of SWDs	mean duration of SWDs, sec	number of SWDs	mean duration of SWDs, sec
2-3 (n = 6)	2.2 ± 1.1	1.2 ± 0.1	–	–
6-7 (n = 7)	11.6 ± 2.5	4.3 ± 0.2	0.25 ± .01	1.3 ± .1

Footnote. Means ± s.e.m. are shown.

TABLE 2. Concentration of TNF-a and IL-1b in the Serum and Brain Tissue of 2, 4, and 6-Months-Old WAG/Rij and ACI Rats

Animal strain	Indices under investigation	Age of animals, months		
		2	4	6
WAG/Rij	TNF-a, serum	6.31 ± 2.0** (n = 12)	2.47 ± 0.89 (n = 13)	1.84 ± 0.94 (n = 12)
	TNF-a, brain	63.8 ± 2.6 (n = 11)	78.7 ± 8.4* (n = 11)	67.3 ± 3.2 (n = 12)
	IL-1b, serum	1.59 ± 0.78 (n = 12)	2.58 ± 0.74 (n = 12)	1.04 ± 0.53 (n = 12)
	IL-1b, brain	27.0 ± 2.8 (n = 11)	32.5 ± 3.8 (n = 11)	25.8 ± 1.7 (n = 11)
ACI	TNF-a, serum	0.61 ± 0.34 (n = 12)	3.53 ± 1.50 (n = 12)	1.27 ± 0.53 (n = 13)
	TNF-a, brain	70.3 ± 3.3 (n = 11)	62.2 ± 4.2 (n = 11)	62.3 ± 2.6 (n = 11)
	IL-1b, serum	1.79 ± 0.78 (n = 12)	1.96 ± 1.07 (n = 12)	1.54 ± 0.73 (n = 12)
	IL-1b, brain	30.9 ± 2.8 (n = 11)	31.7 ± 2.7 (n = 10)	29.2 ± 1.8 (n = 12)

Footnotes. The data are expressed in picograms per milligram of the wet brain tissue. Means ± s.e.m. are shown. Cases with **$P < 0.05$ and *$P < 0.01$ indicate significant differences from the age-matched ACI group. Two-factor (age and strain) ANOVA followed by post-hoc tests was used; n is the number of rats in the group.

($P < 0.1$) and only for TNF-α (second-order interaction, $P < 0.06$).

Experimental Series II. The effect of tiagabine in combination with IL-1β on SWDs is presented in Fig. 4. IL-1β enhanced the intensity of SWDs marginally ($P < 0.1$) in the 1st h after injection and significantly in the 2nd h compared to the effect of BSA injections (335 ± 22.9 *vs* 160 ± 14.8, t = 2.56, df = 14, $P < 0.05$). There were no significant effects in the 3rd and 4th h; in the 5th h, the IL-1β group showed less SWDs than the BSA group (t = 2.71, df = 14, $P < 0.01$) Tiagabine enhanced SWDs in both groups immediately after its administration (t = 4.03, df = 7, $P < 0.01$ and t = 8.55, df = 7, $P < 0.001$) for the BSA and IL-1β groups, respectively. However, the increase following administration of tiagabine was higher in the IL-1β group than that in the BSA (t = 2.79, df = 14, $P <$ < 0.05). The effect of tiagabine was weaker in the 2nd h and disappeared in the 3rd h, and the intergroup differences were no longer significant.

Experimental Series III. EEG recordings were made in young (2 to 3 months) and older (6 to 7 months) rats. The number of SWDs per group and animal age are presented in Table 2. Three effects were found, a main effect for strain, age, and strain × × age interaction. The latter demonstrates that SWD activity increases when rats are getting older, but noticeably more in WAG/Rij rats than in ACI animals.

The data on the cytokine concentrations are presented in Table 2. The overall MANOVA showed a strain × age effect (F = 2.29, df = 8,102, $P < 0.05$). Univariate tests showed a significant strain effect (F = 4.34, df = 1,53, $P < 0.05$; WAG/Rij > ACI) and an interaction between strain and age (F = 5.43, df = = 2,53, $P < 0.01$) for TNF-α serum levels and a similar interaction for TNF-α in the brain (F = 5.29, df = = 2,53, $P < 0.01$). Subsequent post-hoc tests showed that the plasma levels were higher in 2-month-old

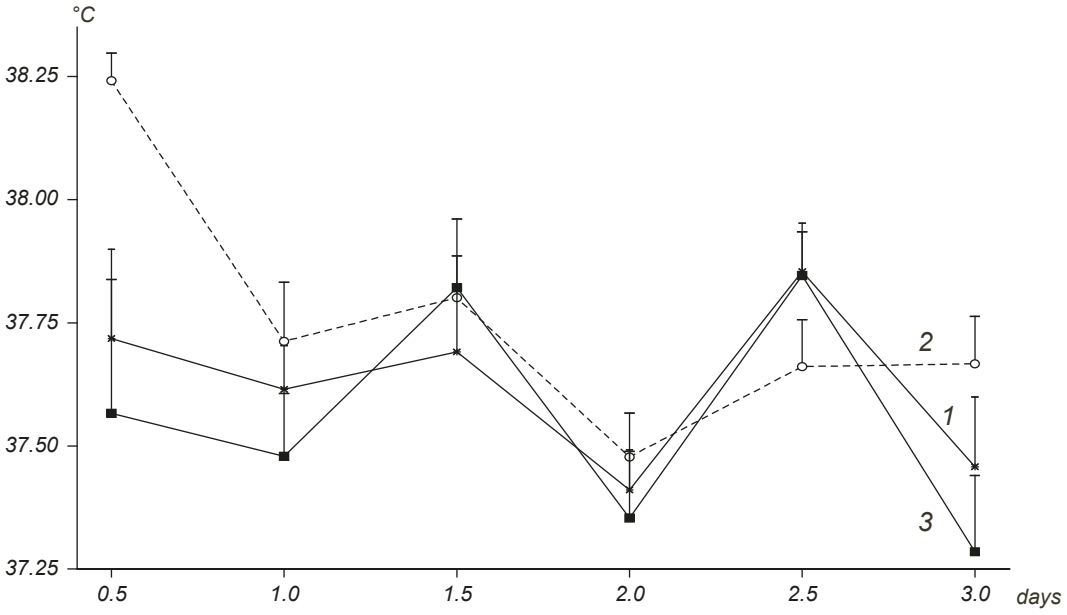

Fig. 3. Rectal temperature (°C) of the WAG/Rij rats measured for 3 days. Note the high temperature of the rats 4 h after TNF-α injection reflecting a hyperthermic response. Significant effects were noticed for days ($P < 0.05$; D1 > D2, D3), for time of day ($P < 0.001$; evening, 17.00 p.m. > morning, 9.00 a.m.), and tendency for a second-order interaction ($P < 0.06$). Altogether, the data demonstrate that the temperature is highest after administration of TNF-α, but only at the first measurement (at the end of the 1st day, several hours after injection). Other designations are the same as in Fig. 1.

WAG/Rij rats *vs* age-matched ACI animals, and brain concentrations tended to be higher in 4-month-old WAG/Rij rats ($P < 0.1$). There were no differences in the 6-month-old rat group.

DISCUSSION

The main goal of this study was to establish a relationship between the cytokines IL-1β and TNF-α and absence epilepsy in a genetic epilepsy model, the WAG/Rij strain of rats. The outcomes of the experimental series I, where TNF-α and IL-1β were administered i.p. and the effects on SWDs were determined within a 72-h-long period, showed an immediate and short-lasting increase in SWDs after IL-1β and a delayed increase in SWDs after TNF-α. The i.p. route of IL-1β and TNF-α administration was chosen since it was found that cytokines reach the CNS directly by crossing at leaky areas of the blood-brain barrier (BBB) through the circumventricular organs even under healthy (basal) conditions [15, 25]. In agreement with the data obtained in models of focal onset, fever, or infection-related models [4] and in the same genetic model after administration of LPS [6], IL-1β aggravates absence seizures. Also, the latency of generalized seizures induced by benzylpenicillin became shorter, while the severity of seizures increased [34]. Our findings demonstrate also that the acute effects found after bacterial LPS introduction were not due to TNF-α but due to either IL-1β or other non-tested cytokines. The effects of IL-1β on SWDs occurred rather rapidly, in the first few hours after its peripheral administration. Their fast onset suggests that these effects are direct, while the effects after LPSs occurred after 90 to 270 min. It is known that IL-1β induces its own synthesis, and the synthesis of IL-6 and TNF-α in the CNS is realized mainly in astrocytes and microglia [35]. It is not known whether IL-6 modulates the SWD manifestation; however, TNF-α had only a delayed effect. Increase in the SWD intensity occurred only 7 to 18 h after its administration. Interestingly, LPS exerts time-dependent effects on the production of TNF-α; in a rat glial cell culture, this factor reaches its peak concentration 6 h after the treatment [36]. Since a direct effect on SWDs does not occur after TNF-α, some other physiological or hormonal mechanism might be at work here. Interestingly, the bioavailability and gene expression of TNF-α has a circadian rhythm with peak levels at the beginning of the light period [28]. At specifically these hours of the 24 h light/

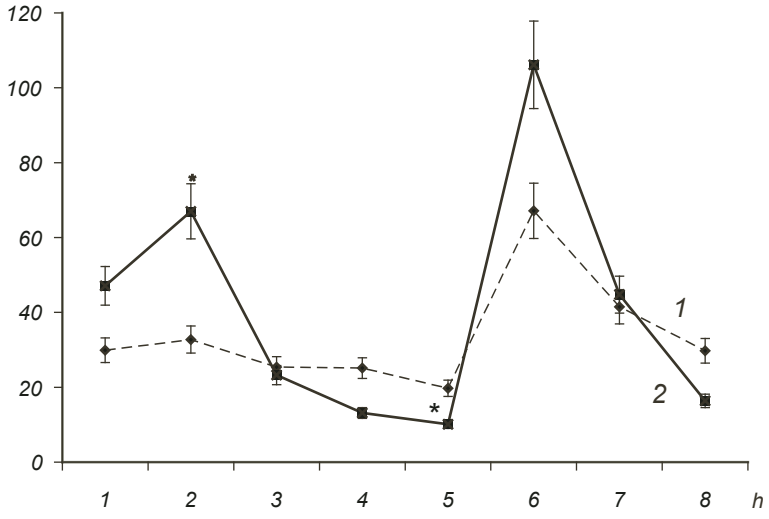

Fig. 4. Number of spike-wave discharges (SWDs) per 1 h after injections of IL-1β and bovine serum albumin (BSA) followed by administration of tiagabine (2 and 1, respectively) on the 6th h. Note that IL-1β enhances the number of SWDs on the 2nd h after injection, even when IL-1β is no longer active; a differential tiagabine-induced increase in SWDs is found. Tiagabine increases SWDs, but the increase is higher in the IL-1β group. $*P < 0.05$.

dark cycle, the number of SWDs is usually quite low [31]. These two facts suggest an inverse relationship between the number of SWDs and the TNF-α level. We consider this another argument for our suggestion that TNF-α itself is not responsible for the increase observed 7-18 h after its administration. Probably, this is another factor, perhaps IL-1β itself, since this interleukin manifests SWD-enhancing effects. We conclude that the effects of TNF-α on SWDs are not directly mediated by TNF-α. We speculate that TNF-α emerges through IL-1β.

It is also quite possible that the presently found increase in the frequency of SWDs is due to nonspecific effects induced by the cytokines, such as their effects on the HPA axis, fever responses, and somnogenic effects. Some of these factors exert well-described effects on the occurrence of SWDs. Corticosteroids and stress facilitate the occurrence of SWDs [37, 38]; it should also be taken into account that corticosteroids induce β-endorphin release. β-Endorphin is known to enhance the mu- and delta-opioid receptors. The mu-opioid receptor agonist DAMGO increased SWDs in WAG/Rij rats [39]. A second major candidate that might explain the increase in the SWD intensity is body temperature. TNF-α and interleukins increase the body temperature after systemic injections [40]; an increase of 0.5ºC was found 4 h after injection of TNF-α, but the analogous effect was absent after IL-1β. Therefore, it is not likely that the increase in SWDs after IL-1β can be ascribed to its pyrogenic effect. The lack of a clear increase in the body temperature after IL-1β might be due to the smaller dose we used (2,000 ng/kg injected i.p. *vs* 3,000 ng/rat injected i.v.).

The behavior of the rats was changed immediately after administration of both IL-1β and TNF-α; these agents induced more passive behavior. This fact demonstrates that both compounds are biologically effective in this respect. Intensification of passive behavior is also indicative of an increase in sleep duration. There is convincing evidence that IL-1β and TNF-α are involved in the regulation of sleep, since systemic administration of both substances induces an increase in the amount of non-REM sleep. This increase manifests itself within 1 h after injection and persists 4 to 10 h [28]. Considering the intimate relationship between the relative amount of passive behavior (including drowsiness and light slow-wave sleep) and the number of SWDs [41], it is possible that a rise in SWDs found after administration of IL-1β is due to an increase in passive behavior and/or sleep. We have no behavioral data during the light episode. Therefore, it still needs to be determined whether the increase of SWDs after TNF-α in the light period (6 h) after administration is accompanied by changes in the behavior, or whether the increase in SWDs is due to more passive behavior than that in the two other groups.

Our own data demonstrated that Il-1β increases the effects of the GABA reuptake blocker tiagabine. It is well known that GABA mimetics, including tiagabine, aggravate SWD phenomena when administered systemically [30]. An enhancement of postsynaptic GABA-ergic potentials was found in a chronic LPS inflammation model [42]. Therefore, it is proposed here that the action of this GABA-mimetic anticonvulsant and proabsence drug is aggravated by IL-1β at a time point when IL-1β is no longer effective or even suppresses SWDs. This suggests that this cytokine may affect the efficacy of the above

antiepileptic drug. The duration of the action of tiagabine (about 1 h) is in complete agreement with outcomes of an earlier study in WAG/Rij rats [29]. It was also confirmed that 6-month-old WAG/Rij rats have a large number of SWDs, while at the age of 2 months they have none. The EEG pattern of some of these young WAG/Rij rats was characterized by 5-Hz oscillations far from fitting the criteria for genuine SWDs. However, these oscillations might develop into genuine SWDs when rats grew older. The ACI rats hardly showed any SWD at either age. These data are in agreement with earlier observations on the age-dependent increase in WAG/Rij rats from the age of 3 months onwards and a much lower incidence in ACI rats [21, 23, 24, 43].

Higher concentrations of TNF-α were found in the blood plasma of WAG/Rij *vs* those in ACI rats (irrespectively of age), next to an age-dependent strain differences. The serum level of TNF-α was enhanced in young WAG/Rij rats preceding the onset of SWDs. The brain concentration tended to increase in WAG/Rij rats when the animals reached 4 months. In the case where SWDs were fully developed at 6 months of age, there were no longer differences between the two strains. Therefore, in general, there is some evidence that TNF-α might be involved in the pathogenesis of absence seizures in WAG/Rij rats. The high concentration of TNF-α in pre-epileptic or pre-symptomatic rats and somewhat increased brain concentrations in 4-month-old rats might be considered a form of neuroprotection towards changes from a pro-epileptic to an epileptiformic brain. Neuroprotective effects for this cytokine have been proposed by Peltola et al. [44, 45] and Marchetti et al. [46]. Also, Plata-Salaman et al. [13] proposed that cytokines play a role in adaptive mechanisms associated with generalized seizure activity with implications for neuroprotection. The high concentration of TNF-α in young, pre-symptomatic WAG/Rij rats, together with the age-dependent increase in SWDs and the age-dependent decrease in TNF-α, also underline the opposite relation between this cytokine and SWDs.

Acknowledgments. This research was supported by INTAS grant No. 2037. The authors would like to thank Hans Krijnen, Saskia Hermeling, and Elly Willems van Bree for biotechnical and biochemical assistance.

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