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Antibodies to S100 proteins have anxiolytic-like activity at ultra-low doses in the adult rat

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Abstract

S100 proteins are small calcium-binding proteins interacting with numerous intra- and extra cellular targets involved in diverse physiological functions. In particular, S100 proteins may be involved in the regulation of anxiety-related behaviour. In the present study, the effects of affinity-purified antibodies to S100 proteins administered orally at ultra-low doses were evaluated in pre-clinical tests for anxiolytic-like activity in the adult rat. In the Vogel conflict test in the rat, antibodies to S100 proteins increased punished drinking (anti-conflict effect) at 5 and 7.5 mL kg^{-1}, but not at 2.5 or 10 mL kg^{-1}. Antibodies to S100 proteins increased the percentage of entries into the open arms of an elevated plus-maze at 10 mL kg^{-1}, but not at lower doses. Taken together, these results indicate the presence of anxiolytic-like activity for antibodies to S100 proteins over the dose range 5–10 mL kg^{-1} in the adult rat.

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Introduction

S100 proteins belong to a large family of EF-hand calcium-binding proteins (Donato 2001). In general, an increase in calcium concentration induces calcium binding to the EF-hand motif and modification of the conformation of S100 proteins with ensuing interaction with their target proteins (Santamaria-Kisiel et al 2006). The S100 family of proteins comprises more than 25 different members, each of them being characterized by specific tissue and cell expression, molecular composition (homo- or hetero-dimers) and target proteins (Donato 2001).

S100 proteins are involved in cell division, growth, differentiation and survival. The majority of the functions of S100 proteins are calcium-dependent, although calcium-independent functions have also been described (Santamaria-Kisiel et al 2006). Calcium-dependent actions of S100 proteins include regulation of various enzymatic activities, cytoskeletal proteins, signal transduction and calcium homeostasis. This broad spectrum of actions explains the implication of S100 proteins in various physiological and pathological conditions such as inflammation, arthritis and inflammation-associated cancer (Gebhardt et al 2006; Senolt et al 2006; Foell et al 2007).

In the nervous system, S100 proteins have intracellular and extracellular actions (Donato 2003). S100 proteins are involved in the regulation of synaptic vesicle trafficking (Benfenati et al 2004), cell survival and differentiation (Arcuri et al 2005). At the extracellular level, S100 proteins interact with the receptor for advanced glycation end products, although other receptors may also be involved (Donato 2003). Glial cells release S100 proteins after brain insults, perhaps explaining increased serum S100 protein levels in several pathologies, including stroke, head injury and neurodegenerative diseases (Donato 2001). This led to the assumption that serum S100 protein levels may be a marker for cerebral damage/injury (Lomas & Dunning 2005; Korfias et al 2006; Stroick et al 2006), in particular the event of disruption to the blood–brain barrier (Kleindienst & Ross 2006). Depending on their extracellular concentrations, S100 proteins display divergent effects in cultured neuronal and glial cells,

with trophic effects in the nanomolar range but toxic effects in the micromolar range (Adami et al 2004; Reali et al 2005; Businaro et al 2006). Similar dose-dependent effects of S100 proteins have been described on learning and memory in rodents (Donato 2001). Also, antibodies to S100 proteins impair long-term potentiation (Rebaudo et al 2000) and induce amnesia for passive avoidance in chicks (O'Dowd et al 1997) at high doses, but have opposite effects at ultra-low doses (Epstein et al 2003, 2006). The detrimental effects of high brain levels of S100 protein on memory function suggests that the increased levels of S100 protein measured in the brain of patients may be causally linked to some cognitive deficits associated with Alzheimer's disease (Korfias et al 2006).

Several lines of evidence suggest a role for S100 proteins in anxiety. Transgenic mice over-expressing S100 proteins (S-100 β mice) display deficits in habituation to novel situations in the open field, novel arm exploration in the Y-maze and novel object gnawing tests (Bell et al 2003). In addition, S-100 β mice are less sensitive than wild-type mice to the anxiogenic-like effects of buspirone in the hole board test. These effects were not associated with increased exploration of the light compartment in the light/dark box test, suggesting that S-100 β mice display deficits in habituation to novelty and harm avoidance, although they are not associated with a drastic disruption of anxiety-related behaviour (Bell et al 2003). Conversely, S100 protein knockout mice (S100A1KO mice) display decreased anxiety-related behaviour in avoidance-approach tests, suggesting a role for S100 proteins in the modulation of fear and anxiety (Ackermann et al 2006). Restraint stress increases serum S100 protein levels (Scaccianoce et al 2004) and exposure to a predator odour transiently increases cerebrospinal fluid S100 protein levels in the adult rat (Margis et al 2004). Serum S100b protein concentrations are increased in parallel with anxiety-like behaviour in adult male rats following exposure to electric shocks and weekly sessions of situational reminders (Diehl et al 2007). Among the different members of the S100 family of proteins, S100A10 (also named P11) seems most closely linked to depression and anxiety-related behaviour (Svenningsson & Greengard 2007). Mice over-expressing S100A10 in forebrain neurons display decreased thigmotaxis and increased activity in the open field test, whereas S100A10 knockout mice have an opposite pattern of activity in the same test (Svenningsson et al 2006).

In humans, serum S100 protein levels are inversely correlated with neuropsychological performance, including measures of anxiety, in patients undergoing cardiac surgery (Kilminster et al 1999). In contrast, serum S100 β protein levels are not correlated with neurological performance after cardiopulmonary bypass (Westaby et al 2000). It has also been shown that serum S100 β protein levels are correlated with global functioning score in alcohol-dependent individuals (Liappas et al 2006). Unfortunately, the above studies did not specifically evaluate the relationships between S100 proteins and anxiety, since they used global evaluation scales for neuropsychological functions and global functioning of patients. Recent reports on ultra-low doses of S100 antibodies have aroused interest in the studies of their effects in different areas of pharmacology (Bellavite et al 2006a). Antibodies at ultra-low doses have also been shown to have potential therapeutic use (Epstein 2003).

In the present study, we evaluated the effects of affinity-purified antibodies to S100 proteins (further described as S100 antibodies) administered orally at ultra-low doses (homoeopathic dilutions) in pre-clinical tests for anxiety-related behaviour. We used two of the most widely used tests for detection of anxiolytic-like activity, the Vogel conflict test (Vogel et al 1971) and the elevated plus-maze test (Handley & Mithani 1984) in the adult rat.

Materials and Methods

Animals

Experiments were carried out on male Rj:Wistar (Han) rats, 160–227 g, 6–7 weeks old at the start of the experiments (Elevage Janvier, 53940 Le Genest-Saint-Isle, France). Animals were housed in groups of five in macrolon cages ($41 \times 25 \times 18$ cm) on wood litter with free access to food and water prior to testing. The animal house was maintained under artificial lighting (12 h) between 0700 and 1900 hours at a controlled ambient temperature of 21 ± 3°C, and relative humidity of 30–80%. All procedures were performed in accordance with a currently valid licence for experiments on vertebrate animals, issued by the French Ministry for Agriculture and Fisheries and with a currently valid test facility accreditation for experimentation.

In each experiment, 10 rats were studied per group. Behavioural testing was performed between 1000 and 1600 hours. Animals were killed at the end of the experiments by exposure to O_2/CO_2 (20%/80%) followed by CO_2.

Vogel conflict test in the rat

The method followed that described by Vogel et al (1971). Rats were deprived of water for approximately 48 h before the test and were then placed individually into a transparent Plexiglas enclosure ($15 \times 32 \times 34$ cm) with a floor consisting of stainless steel bars (0.4 cm) spaced 1 cm apart. The back wall of the enclosure was made of opaque Plexiglas, thereby concealing the observer from the experimental animal. In the centre of the opposite wall, 5 cm above the floor, a metal water spout protruded into the cage and was connected to one pole of a shock generator (Apelex: Type 011346, Bagneux, France). The other pole of the shock generator was connected to the metal grid floor.

The rat was left to explore until it found the water spout. Then, every time it drank, it received a mild electric shock (1.7 mA) 2 s after it started drinking. The number of shocks received was manually recorded for 3 min from the time the animal first started drinking.

Elevated plus-maze test in the rat

The method followed that described by Handley & Mithani (1984). The maze consisted of four arms of equal length and width (50×10 cm) arranged in the form of a cross. Two opposite arms were enclosed by 40-cm high walls (closed

arms). The two other arms had no walls (open arms). The maze was raised 65 cm above the floor. A rat was placed in the centre of the plus-maze (head oriented in direction of open arms) and left to explore. The number of entries (four paws in a given arm) into the open and closed arms and the time spent in the open arms were manually recorded during a 5-min test.

Treatments

S100 antibodies were supplied as ready-to-use solutions by NPF Materia Medica Holding, Moscow, Russia. Polyclonal antibodies to S100 protein were kindly supplied by Dr S. M. Sviridov (Institute of Cytology and Genetics SB RAMS, Novosibirsk, Russia). Ultra-low doses of polyclonal antibodies to S100 protein (initial concentration of antibodies before dilution $2\,mg\,mL^{-1}$) were obtained using routine homoeopathic methods as described in the German Pharmacopeia (2005). Antibodies were raised against S100 protein, which consists of A and B subunits. S100A contains α and β units; S100B contains two β units. Procedures of S100 purification and antibody preparation have been described previously (Starostina et al 1981). All dilutions were prepared in glass vials. Antibodies to S100 protein ($2\,mg\,mL^{-1}$) were mixed with the solvent in the ratio 1:99 and shaken manually at 20 succussions per minute for 1 min to produce the C1 dilution. All subsequent dilutions consisted of one part of the previous dilution to 99 parts solvent, with succussion between each dilution. Solutions were prepared in sterile conditions, avoiding direct intense light, and were stored at room temperature. Distilled water was the solvent. The mixture of homoeopathic water dilutions (C12+C30+C200 (1+1+1)) was administered to animals at a volume of 2.5 (single dose experiments) or 5, 7.5 and $10\,mL\,kg^{-1}$ (dose–response experiments). In all groups the same mixture of water dilutions (C12+C30+C200) was used. Ultra-low doses of antibodies are water dilutions, so the liquid form of the active substance makes it impossible to express doses in 'standard' units. In pre-clinical studies, doses are expressed in $mL\,kg^{-1}$ instead of $mg\,kg^{-1}$. The liquid form of ultra-low doses limits the range of doses due the maximal permitted volume of oral administration. A dose of $10\,mL\,kg^{-1}$ was selected as the maximal dose in this study as it is approximately half of the maximal permitted volume of oral administration for rats of 160–220 g ($4\,mL/rat \approx 20\,mL\,kg^{-1}$). To study the dose–effect relationship the other doses were chosen over the range of 0–$10\,mL\,kg^{-1}$. Diazepam was obtained from the Coopération Pharmaceutique Française (Meulen, France) and clobazam was from Sanofi-Synthélabo (Quétigny, France). Hydroxypropylmethylcellulose was obtained from Sigma (Saint Quentin Fallavier, France) and distilled water was from Laboratoires Aguettant (Lyon, France). Diazepam and clobazam are widely used benzodiazepines with anxiolytic effects at the doses used in the present study (Thiebot et al 1976; Millan & Brocco 2003).

All substances were stored in a dry, dark, controlled access area and maintained at a controlled ambient temperature of $20 \pm 3°C$. The vehicle solutions/suspensions were stored at 4°C. Distilled water was used as vehicle. Diazepam and clobazam were dispersed in 0.2% hydroxypropylmethylcellulose in distilled water. The doses of S100 antibodies are expressed in $mL\,kg^{-1}$ of supplied substance. The doses of diazepam and clobazam are expressed in $mg\,kg^{-1}$ of base. Drugs were administered between 1000 and 1100 hours. For single-dose experiments, S100 antibodies ($2.5\,mL\,kg^{-1}$) and diazepam ($2\,mL\,kg^{-1}$) were administered orally once daily for 4 days (a.m.) and then 60 min before the test on Day 5, and compared with a vehicle control group (receiving five administrations of distilled water). Clobazam ($64\,mg\,kg^{-1}$ p.o.), administered acutely 60 min before the test on Day 5 (preceded by one daily administration of distilled water for 4 days), was used as the reference substance.

Dose–response experiments

S100 antibodies were evaluated at three different doses (5, 7.5 and $10\,mL\,kg^{-1}$), administered orally once daily for 4 days (a.m.) and then 60 min before the test on Day 5, and compared with a vehicle control group (receiving five administrations of distilled water). Each experiment included a group treated with diazepam and a group treated with clobazam, as described above.

Because of the different volumes of administration, the experiment was not fully blind. Nevertheless, all groups were coded. Therefore, when the administration volume was identical, the experimenter was blind regarding the nature of vehicle, test, comparison and reference substances. Each experiment included the same number of rats per group. All groups were run in parallel and the treatments were randomized among subjects.

Statistical analysis

Results are presented as means ± s.e.m. for 10 animals per group and as the percent change from appropriate control. Data were analysed by one-way analysis of variance followed by post-hoc unpaired Student's t-test comparing treated groups with vehicle control. All statistical calculations were performed using commercial software (Microsoft Excel, GB Stat version 6.5). Differences were considered statistically significant when the null hypothesis could be rejected at an α risk value of less than 0.05.

Results

Single-dose experiments

Vogel conflict test

The treatments significantly modified the number of shocks received, as indicated by one-way analysis of variance ($F(3, 36) = 3.999$, $P < 0.001$). As shown in Table 1, S100 antibodies ($2.5\,mL\,kg^{-1}$), administered orally once daily (a.m.) from Day 1 to Day 4 and then 60 min before the test on Day 5, did not affect the number of shocks received (punished drinking), as compared with vehicle controls. Diazepam ($2\,mg\,kg^{-1}$), administered under the same experimental conditions, tended to increase the number of shocks received (+77%, $P = 0.0524$). Clobazam ($64\,mg\,kg^{-1}$ p.o.), administered acutely 60 min before the test on Day 5, clearly increased the number of shocks received (+111%, $P < 0.01$).

Table 1 Effect of S100 antibodies at a single-dose, and diazepam and clobazam in the Vogel conflict test in the rat

Treatment	Punished drinking (number of shocks) 1.7 mA		
	Mean ± s.e.m.	*P* value	% Change from control
Vehicle	3.5 ± 0.6	–	–
S100 antibodies (2.5 mL kg^{-1})	4.2 ± 0.6 NS	0.4380	+20%
Diazepam (2 mg kg^{-1})	6.2 ± 1.2 NS	0.0524	+77%
Clobazam (64 mg kg^{-1} p.o.)	7.4 ± 1.1**	0.0049	+111%

S100 antibodies and diazepam were administered (at 2.5 mL kg^{-1}) orally once daily (a.m.) from Day 1 to Day 4 and then 60 min before the test on Day 5, while clobazam (5 mL kg^{-1}) was administered acutely 60 min before the test on Day 5 (preceded by vehicle administration orally once daily (a.m.) from Day 1 to Day 4). Data are shown as mean ± s.e.m. (n = 10 rats per group). Analysis of variance showed a significant effect of treatments on the number of shocks received (punished drinking) ($P < 0.05$). **$P < 0.01$ (Student's *t*-test) compared with vehicle controls. NS, not significant.

Elevated plus-maze test

The treatments significantly modified the total number of entries, the percentage of entries into the open arms and the time spent in the open arms ($F(3, 36) = 9.127$, $F(3, 36) = 20.203$ and $F(3, 36) = 16.854$, respectively, $P < 0.001$). As shown in Table 2, S100 antibodies (2.5 mL kg^{-1}), administered orally once daily (a.m.) from Day 1 to Day 4 and then 60 min before the test on Day 5, did not affect the total number of entries, the percentage of entries or the time spent in the open arms, as compared with vehicle controls. Diazepam (2 mg kg^{-1}), administered under the same experimental conditions, significantly increased the total number of entries (+35%, $P < 0.05$). It clearly increased the percentage of entries and the time spent in the open arms (+70%, $P < 0.01$ and +85%, $P < 0.05$, respectively). Clobazam (64 mg kg^{-1} p.o.), administered acutely 60 min before the test on Day 5, also increased the total number of entries (+60%, $P < 0.01$). It markedly increased the percentage of entries and the time spent in the open arms (+174% and +242%, respectively, $P < 0.001$).

Taken together, the results of the single-dose experiments suggested the absence of a significant anxiolytic- or anxiogenic-like activity for S100 antibodies at 2.5 mL kg^{-1} in the Vogel conflict test and the elevated plus-maze test in the rat. The following dose–response experiments were performed to evaluate a higher range of doses in the same two tests.

Dose–response experiments

Vogel conflict test

The treatments significantly modified the number of shocks received ($F(5, 52) = 2.412$, $P < 0.05$). As shown in Figure 1, S100 antibodies (5 and 7.5 mL kg^{-1}), administered orally once daily (a.m.) from Day 1 to Day 4 and then 60 min before the test on Day 5, dose-dependently increased the number of shocks received, as compared with vehicle controls (+38%, $P = 0.0619$ and +53%, $P < 0.05$, respectively).

Table 2 Effect of S100 antibodies at a single-dose, and diazepam and clobazam in the elevated plus-maze test in the rat

Treatment	Total number of entries			% Entries into open arms			Time spent in open arms (s)		
	Mean ± s.e.m.	*P* value	% Change from control	Mean ± s.e.m.	*P* value	% Change from control	Mean ± s.e.m.	*P* value	% Change from control
Vehicle	13.1 ± 1.0	–	–	18.0 ± 3.1	–	–	26.6 ± 6.0	–	–
S100 antibodies (2.5 mL kg^{-1})	10.5 ± 1.1 NS	0.1024	−20%	17.1 ± 3.5 NS	0.8498	−5%	14.6 ± 4.4 NS	0.1236	−45%
Diazepam (2 mg kg^{-1})	17.7 ± 1.8*	0.0365	+35%	30.6 ± 3.0**	0.0091	+70%	49.1 ± 7.4*	0.0288	+85%
Clobazam (64 mg kg^{-1} p.o.)	21.0 ± 2.0**	0.0027	+60%	49.4 ± 3.7***	< 0.0001	+174%	90.9 ± 12.6***	0.0002	+242%

Treatment was the same as described for Table 1. Data are shown as mean ± s.e.m. (n = 10 rats per group). Analysis of variance showed a significant effect of treatments on the total number of entries, the percentage of entries into the open arms and the time spent in the open arms ($P < 0.001$). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (Student's *t*-test) compared with vehicle controls. NS, not significant.

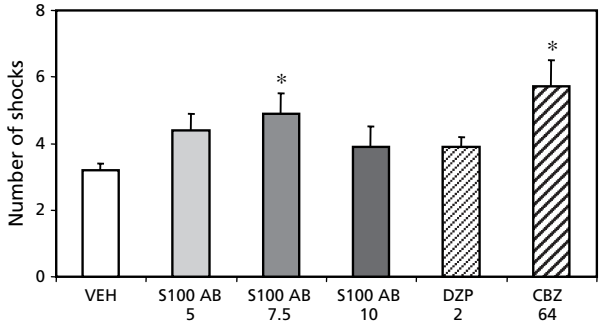

Figure 1 Effect of S100 antibodies (S100 AB), diazepam (DZP) and clobazam (CBZ) in the Vogel conflict test in the rat. The experimental conditions were the same as described for Table 1 except that S100 antibodies were administered at 5, 7.5 or 10 mL kg^{-1}, and diazepam and clobazam were administered at 5 mL kg^{-1}. Data are shown as mean ± s.e.m. (n = 10 rats per group). One-way analysis of variance showed a significant effect of treatments on the number of shocks received (punished drinking) ($P < 0.05$). $^{*}P < 0.05$ (Student's *t*-test) compared with vehicle (VEH) controls.

S100 antibodies had no clear effect at 10 mL kg^{-1}. Diazepam (2 mg kg^{-1}), administered under the same experimental conditions, did not affect the number of shocks received. Clobazam (64 mg kg^{-1} p.o.), administered acutely 60 min before the test on Day 5, significantly increased the number of shocks received (+78%, $P < 0.05$).

Elevated plus-maze test

The treatments significantly modified the total number of entries, the percentage of entries into the open arms and the time spent in the open arms ($F(5, 54) = 31.244$, $F(5, 53) = 12.336$ and $F(5, 54) = 28.199$, respectively, $P < 0.001$). As shown in Figure 2, S100 antibodies (5, 7.5 and 10 mL kg^{-1}), administered orally once daily (a.m.) from Day 1 to Day 4 and then 60 min before the test on Day 5, did not affect the total number of entries, as compared with vehicle controls. S100 antibodies significantly increased the percentage of entries into open arms at 10 mL kg^{-1} (+71%, $P < 0.05$), but had no effect at lower doses. S100 antibodies did not affect the time spent in open arms. Diazepam (2 mg kg^{-1}), administered under the same experimental conditions, significantly increased the total number of entries (+48%, $P < 0.05$). It also significantly increased the percentage of entries and the time spent in the open arms (+96% and +149%, respectively, $P < 0.05$). Clobazam (64 mg kg^{-1} p.o.), administered acutely 60 min before the test on Day 5, clearly increased the total number of entries (+178%, $P < 0.001$). It markedly increased the percentage of entries and the time spent in the open arms, as compared with vehicle controls (+342% and +850%, respectively, $P < 0.001$).

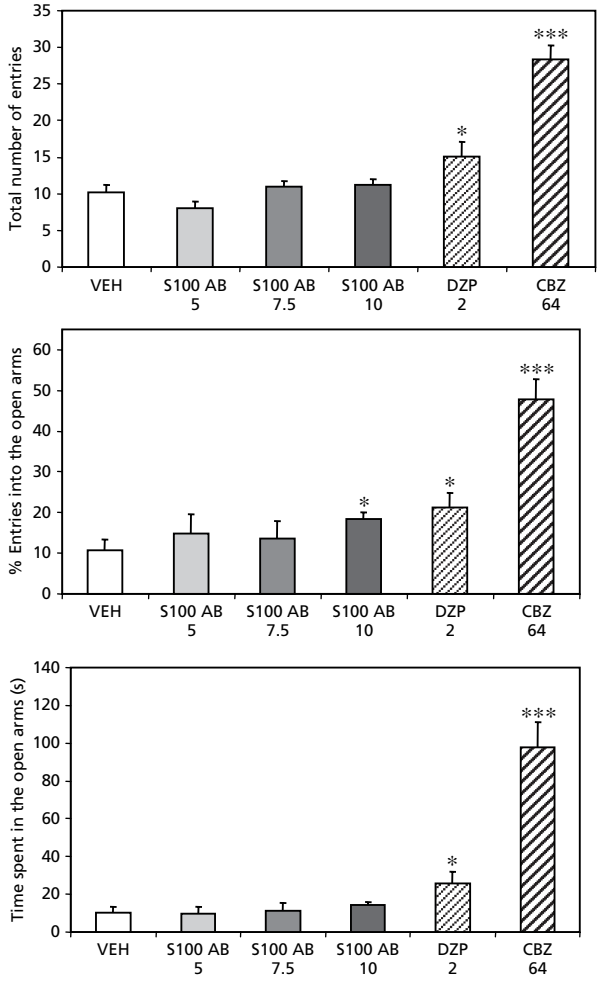

Figure 2 Effect of S100 antibodies (S100 AB), diazepam (DZP) and clobazam (CBZ) in the elevated plus-maze test in the rat. The experimental conditions were the same as described for Table 2 except that S100 antibodies were administered at 5, 7.5 or 10 mL kg^{-1}, and diazepam and clobazam were administered at 5 mL kg^{-1}. Data are shown as mean ± s.e.m. (n = 10 rats per group). One-way analysis of variance showed a significant effect of treatments on the total number of entries, the percentage of entries into the open arms and the time spent in the open arms ($P < 0.001$). $^{*}P < 0.05$ and $^{***}P < 0.001$ (Student's *t*-test) compared with vehicle (VEH) controls.

Discussion

The results indicate the presence of anxiolytic-like activity for S100 antibodies over the dose range 5–10 mL kg^{-1}, but not at 2.5 mL kg^{-1}, in the Vogel conflict and the elevated plus-maze tests in the rat.

Clinical data linking S100 proteins to anxiety are sparse and do not allow a precise role for S100 proteins in anxiety to be defined. Available pre-clinical data tend to suggest that over-expression of S100 proteins is generally associated with decreased anxiety-related behaviours (Bell et al 2003; Ackermann et al 2006; Svenningsson & Greengard 2007). In contrast, administration of ultra-low doses of S100 antibodies at 10 mL kg^{-1} reduces the anxiogenic effects of acute stress (electric shock) administered before evaluation of anxiety-related behaviour in the elevated plus-maze test in the rat (Loskutova et al 2003). The anxiolytic-like effects of ultra-low doses of S100 antibodies were also demonstrated in

the absence of sedative or myorelaxant effects (Voronina et al 2006).

The Vogel conflict test is based on the conflict between the drive of thirsty rats and avoidance of an aversive electric shock (Vogel et al 1971). Anxiolytics increase punished drinking (i.e. have an anti-conflict effect). In addition to benzodiazepine-like anxiolytics, the Vogel test also detects the effects of substances acting on serotonergic uptake (Moser et al 1990; Dekeyne et al 2000; Chojnacka-Wojcik et al 2005) and several other pharmacological classes of substances with suspected clinical anxiolytic activity (Millan & Brocco 2003). Taken together, these data suggest that the Vogel test is useful for detecting a wide range of typical and atypical anxiolytics (Castagné et al 2006). In the present study, S100 antibodies increased punished drinking at 5 and 7.5 $mL\,kg^{-1}$, but not at 2.5 or 10 $mL\,kg^{-1}$. Although absence of an effect at 2.5 $mL\,kg^{-1}$ may be explained by an insufficient exposure to S100 antibodies, absence of an effect at 10 $mL\,kg^{-1}$ may be explained by satiation of the animals. Indeed, due to the daily oral administrations, the rats were not totally deprived of water during the 48 h preceding the test. As a result, the rats were less thirsty than after complete deprivation of water and thus were more prone to avoid electric shock. Satiation may increase with the volume of administration, thereby counteracting the effects of S100 antibodies. Although the Vogel conflict test clearly detected the anxiolytic effects of clobazam (64 $mg\,kg^{-1}$) after acute administration, it failed to detect a statistically significant activity of diazepam (2 $mg\,kg^{-1}$) after repeated administration. The lack of activity of diazepam cannot be explained by an anti-thirst effect of its vehicle since vehicle controls, which displayed a low number of punished drinking, received the same volume of administration. It is more likely that the dose of diazepam was too low in the present study or that tolerance to its effects occurred after 5 days of administrations.

The elevated plus-maze test is based on the spontaneous aversion of rodents to open environments (Handley & Mithani 1984) (Pellow et al 1985). During the test, rats are placed in a cross-shaped maze with two closed arms providing a safe environment and two open arms providing a potentially dangerous (anxiogenic) environment. A normal rat spends most of its time in the closed arms. It is considered that the behaviour of the animals reflects a conflict between the drive for exploration of a new environment (the whole maze) and the natural tendency to avoid potentially dangerous environments (open arms). It is possible to measure several parameters during the test. The most often used parameters are the time spent in the open arms and the proportion of open arm entries (Moser 1989; Griebel et al 1997). Anxiolytics, and in particular benzodiazepines, increase the relative exploration of open arms (Graeff et al 1998; Castagné et al 2006). Excitatory effects may be associated with a non-specific increase in exploration of both the open and the closed arms. In contrast, sedative effects are indicated by a decrease in exploration of both the open and closed arms. After acute administration, several monoamine reuptake inhibitors do not display anxiolytic activity in the elevated plus maze test (Castagné et al 2006). Nevertheless, activity of monoamine reuptake inhibitors can be revealed after chronic treatment (File et al 1999), consistent with their use as primary treatment in several anxiety disorders (Nutt 2005). In the present study, S100 antibodies increased the percentage of entries in the open arms at 10 $mL\,kg^{-1}$, but not at lower doses. The higher effective dose of S100 antibodies in the elevated plus-maze as compared with the Vogel conflict test may be explained by differences in the type of anxiety-related behaviour in the two tests but also by intrinsic activity (decrease in thirst) of the vehicle in the Vogel conflict test. The elevated plus-maze test detected anxiolytic effects of clobazam (64 $mg\,kg^{-1}$) after acute administration and of diazepam (2 $mg\,kg^{-1}$) after repeated administration. This shows that under our experimental conditions, the elevated plus-maze test is more sensitive to the effects of diazepam (or less sensitive to tolerance) than the Vogel conflict test after repeated administration.

Conclusion

Our data indicate that repeated daily oral treatment for 5 days with ultra-low doses of affinity-purified antibodies to S100 proteins decreases anxiety-related behaviour in two widely used pre-clinical models for anxiolytic-like activity, the Vogel conflict and the elevated plus-maze tests in the rat. The present results are in accordance with a previous study showing anxiolytic-like activity of ultra-low doses of antibodies to S100 proteins in previously stressed rats (Loskutova et al 2003). Up to now, evidence concerning the effects of ultra-low doses of antibodies to S100 proteins suggests potential anxiolytic-like activity in rodents, although nothing is known of their potential mechanism of action after oral administration. It has been reported that homoeopathic treatments display efficacy in patients affected by anxiety disorders (Davidson et al 1997; Oberbaum et al 2003). Nevertheless, these results have not been confirmed in double-blind studies (Cialdella et al 2001; Baker et al 2003; Bonne et al 2003). Recent reviews also report efficacy of several homeopathic medicines in anxious patients (Sevar 2005; Mathie & Robinson 2006) although a systematic review indicates that more evidence is needed in order to draw firm conclusions (Pilkington et al 2006). An advantage of homoeopathic medicines, and in particular of ultra-low doses of antibodies, is their general lack of side-effects despite efficacy in various models (Bellavite et al 2006a, b). Our positive results together with previous data (Loskutova et al 2003; Voronina et al 2006) provide evidence for potential anxiolytic-like effects of ultra-low doses of affinity-purified antibodies to S100 proteins and suggest they may offer an interesting, low-risk alternative treatment to conventional anxiolytics.

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