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Centrally administered oligodeoxynucleotides in rats: occurrence of non-specific effects

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Abstract

We studied the effects of various intracerebroventricularly administered oligodeoxynucleotides on body temperature, locomotor activity, food intake and water consumption in rats during a 24 h period with a radio-telemetric system. Both complete phosphorothioate oligodeoxynucleotides and end-inverted oligodeoxynucleotides dose-dependently elevated body temperature, suppressed food and fluid intake and inhibited nighttime activity. Apparently these effects do not depend on the nucleotide sequence because antisense and sense arginine vasopressin and oxytocin oligodeoxynucleotides, as well as a missense oligodeoxynucleotide produced comparable changes in the autonomous and behavioral parameters. In control experiments neither contaminants from the chemical synthesis nor endotoxins produced such effects, whereas native DNA from salmon sperm did. Fever and sickness-like behavior in response to missense phosphorothioate oligodeoxynucleotides were accompanied by elevated concentrations of circulating corticosterone and by a marked increase in interleukin 6 mRNA in brain and spleen, indicating that centrally administered oligodeoxynucleotides stimulate the production of pyrogenic inflammatory mediators in both central nervous system and peripheral tissues. Our results indicate that centrally administered oligodeoxynucleotides produce beside their intended sequence-specific effects also transient and sequence-independent effects due to their nucleic acid structure. © 1997 Elsevier Science B.V.

Keywords: Antisense targeting; Oligodeoxynucleotides; Sickness behavior; Interleukin 6; Radiotelemetry; (Rat)

1. Introduction

Antisense oligodeoxynucleotides are efficient tools for down-regulating the expression of single genes by translational arrest and other mechanisms (Neckers and Whitesell, 1993; Stein and Cheng, 1993; Stein, 1996).

For in vivo studies oligodeoxynucleotides are often injected into target tissues or applied topically, rather than being administered systemically. In many experimental settings, oligodeoxynucleotides have been injected either directly into distinct brain regions or into the cerebral ventricles because the brain seems to have a lower nuclease activity than most peripheral tissues (Whitesell et al., 1993; Wahlestedt, 1994). However, the instability of oligodeoxynucleotides in biological fluids and tissues limits their use as pharmacological tools in basic research and as pharmaceuticals. Therefore, various chemically modified oligodeoxynucleotides, e.g., phosphorothioates and oligodeoxynucleotides with end-inverted internucleotidic linkage, have been designed (Uhlmann and Peyman, 1990; Ortigao et al., 1992).

Although it has been shown that oligodeoxynucleotides exhibit a remarkably low toxicity both in vitro (Crooke, 1991) and in vivo when they are administered systemically or topically (Crooke, 1992), more recent studies reported non-specific effects. Thus, sequence-independent effects of oligodeoxynucleotides in cultured primary cells and cell lines have been observed (Perez et al., 1994) and intraperitoneal administration of phosphorothioate oligodeoxynucleotides to mice was associated with toxic hematological and histopathological effects (Sarmiento et al., 1994). Oligodeoxynucleotide-treated mice consumed less food and were described as weak and cachectic; they displayed a hunched posture and ruffled their fur. Furthermore, Galbraith et al. (1994) reported transient hemodynamic changes and complement activation following systemic infusion of phosphorothioate oligodeoxynucleotides in monkeys. These effects were dose-dependent and did not depend on the

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sequence of the applied oligodeoxynucleotides. However, despite the wide use of the antisense technology in the neurosciences, little is known about the central nervous system toxicity of oligodeoxynucleotides. Because putatively toxic effects are difficult to predict, we analyzed body temperature, locomotor activity, food intake, water consumption and circulating corticosterone concentrations as general parameters of the condition of experimental animals after a single centrally administered dose of oligodeoxynucleotides. We chose these parameters because fever, reduced activity, adipsia and anorexia are the most obvious behavioral and pathophysiological alterations, termed sickness behavior, that accompany most pathological conditions associated with inflammatory reactions (Hart, 1988). Since transient fever in response to oligodeoxynucleotide application into the lateral cerebral ventricle was observed in the present study, we examined in addition interleukin 6 expression in the brain. Interleukin 6 concentrations in the brain correlate with the degree of fever (Nijsten et al., 1987; Muramami et al., 1993; Schöbitz et al., 1994b) and therefore the interleukin 6 content of a given tissue is an accurate measure of the extent of an inflammatory response.

2. Materials and methods

2.1. Animals and surgery

Male adult Wistar rats (180–250 g), purchased from a local supplier (Charles River, Sulzfeld, Germany), were housed five per cage until surgery with free access to standard chow and drinking water under conditions of constant temperature (21 ± 2°C) and a 12:12 h light/dark cycle (lights on at 07.00 h). At the end of the experiment, the animals were killed with an overdose of halothane (Hoechst, Frankfurt, Germany). The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body.

Guide cannulas were implanted into the right lateral cerebral ventricle under halothane aesthesia using standard stereotaxic techniques. The position of the cannula relative to bregma was lateral ± 0.9, anterior -1.5 and 3.2 mm below the surface of the skull according to the atlas of Paxinos and Watson (1986). After surgery the rats were housed singly until the experiment, i.e., for one week.

For repeated blood withdrawal, rats were fitted with chronic i.v. catheters in the jugular vein. The animals were handled daily, the i.v. catheters were rinsed and sham injections were performed.

2.2. Oligodeoxynucleotides and DNA

The following oligodeoxynucleotides were used:

1. A complete phosphorothioate arginine vasopressin antisense oligodeoxynucleotide: CAT GGC GAG CAT AGG TGG (Neumann et al., 1994; Skutella et al., 1994).
2. A complete phosphorothioate arginine vasopressin sense oligodeoxynucleotide: CCA CCT ATG CTC GCC ATG (Neumann et al., 1994; Skutella et al., 1994).
3. A complete phosphorothioate oxytocin antisense oligodeoxynucleotide: CAG GCC ATG GCG TTG GTG (Neumann et al., 1994).
4. A complete phosphorothioate oxytocin sense oligodeoxynucleotide: CAC CAA CGC CAT GGC CTG (Neumann et al., 1994).
5. A complete phosphorothioate missense oligodeoxynucleotide, which has little or no homology to any known sequence of the GenBank database: AGG TGA TCA ACC TGA CGC.
6. A missense oligodeoxynucleotide with 3′-3′-inverted internucleotidic linkage: GTT CCA AGT GGT AAT CCG.

Oligodeoxynucleotides 1–5 were obtained from the Laboratorium für Molekulare Biologie (Genzentrum, Martinsried, Germany). Oligodeoxynucleotide 6 was synthesized by Dr. R. Rösch, Sektion Polymere, University of Ulm (Ulm, Germany). Oligodeoxynucleotides 1 to 6 were purified by high-pressure liquid chromatography and Sephadex gel chromatography. Oligodeoxynucleotides were dissolved in sterile pyrogen-free Ringer solution, containing 147.1 mM Na^+, 2.25 mM Ca^{2+}, 4.0 mM K^+ and 155.6 mM Cl^-. 5 μl of each solution was injected intracerebroventricular (i.c.v.) within a period of 2 min. The pH of the injection solutions was approximately 6. The arginine vasopressin antisense oligodeoxynucleotide was submitted to an additional purification step with ion-exchange chromatography (Quiagen™, Diagen, Düsseldorf, Germany) according to the protocol of the manufacturer.

A mock phosphorothioate oligodeoxynucleotide was prepared by Dr. G. Arnold, Laboratorium für Molekulare Biologie, Genzentrum, Martinsried, Germany with standard controlled pore glass synthesis columns. After the first detritylation step the column bound mononucleotide was subjected to 5 rounds of acetylation of the 5′-hydroxyl group to prevent coupling of additional bases. Subsequent synthesis, cleavage and deprotection steps were performed exactly as with complete phosphorothioate oligodeoxynucleotides. The product was high pressure liquid chromatography-purified and the fractions were collected according to the elution time of standard dimethoxytrityl-oligonucleotides. The 5′-acetylmononucleotide is not present in these fractions.

Sheared salmon sperm DNA was purchased from Sigma (Deisenhofen, Germany).

2.3. Measurement of body temperature, locomotor activity, water consumption and food intake

The animals were kept under conditions of constant temperature ($21 \pm 2\%$), humidity (about 40%) and on a

controlled 12:12 h light/dark cycle (lights on at 07.00 h) throughout the experiments. Core body temperature (± 0.1°C) and locomotor activity (expressed in arbitrary units) were monitored in undisturbed rats with a radiotelemetric method using the Dataquest IV system (Data Sciences International, St. Paul, MN, USA). A battery-powered transmitter was implanted into the peritoneal cavity of each animal under halothane aesthesia. After surgery, animals were allowed to recover for two days and to adapt to the cages prior to the measurements. The frequency of the signal emitted by the transmitter was proportional to the animal's body temperature. The signal was transmitted to a receiver underneath the cage and was transferred to and processed by an IBM personal computer. Body temperature was continually recorded at 5 min intervals. Means of these data were analyzed at 1 h intervals.

Locomotor activity was measured by monitoring changes in the strength of the signal from the transmitter, which occurred when the animal moved. Changes in signal strength generated a digital pulse, which was counted by the Dataquest IV system. For rates of motion greater than 1 cm/s the number of pulses depended strictly on the distance the animal moved. Locomotor activity was continually recorded at 5 min intervals. Means of these data were analyzed at 3 h intervals.

The licking rate (expressed in licks/5 min) was monitored with lick sensors (Data Sciences International), which were connected to the drinking nipple and to a ground wire at each cage. Touching the nipple during fluid intake generates a pulse that is received by the Dataquest IV system. The licking rate was recorded at 5 min intervals. Means of these values were analyzed at 6 h intervals.

Food intake was determined by calculating the change in the weight of the food pellets in the animal cages from before injection to 12 h post-injection and from 12 to 24 h post-injection.

2.4. Endotoxin determinations

The endotoxin content of samples containing oligodeoxynucleotides or salmon sperm DNA was determined with the Toxicolor System™ (Seikagaku, Tokyo, Japan). The assay is based on hydrolysis of a chromogenic peptide substrate, containing *p*-nitroaniline, by a clotting enzyme in the *Limulus* amoebocyte lysate. The enzyme is activated by endotoxin. Combined substrate and lysate solutions were incubated at 37°C for 30 min with the nucleic acids or vehicle and then measured in an enzyme-linked immunosorbent assay reader at 405 nm. The samples were measured in duplicate. A standard curve was plotted, using *E. coli* 0111:B4 standard endotoxin solution (50 pg/ml = 0.145 endotoxin units of the US Pharmacopeial Forum per ml), for determination of the amount of endotoxin in the samples. Lipopolysaccharide, *E. coli* 0111:B4, was purchased from Seikagaku. Polymyxin B sulfate was purchased from Fluka (Buchs, Germany).

2.5. Northern blot analysis

3 and 24 h after injection (i.c.v.) of vehicle or oligodeoxynucleotides, separate groups of rats ($n = 6$ per group) were decapitated and the brain and spleen were dissected. As a positive control, rats were injected i.p. with a pyrogenic, non-lethal dose (100 μg/kg) of purified lipopolysaccharide (*E. coli* 0111:B4, Sigma) in a volume of 1 ml 0.9% saline/kg. 3 h after the injection, the animals were decapitated with halothane and the spleen was removed and analyzed for interleukin 6 mRNA.

Total RNA was isolated by the method of Chomczynski and Sacchi (1987). Northern blotting was performed as described by Sambrook et al. (1989) using ^{32}P-labeled cDNA. The probe was rat interleukin 6 cDNA (Northemann et al., 1989), kindly provided by Dr. M. Baumann, Boehringer-Mannheim (Mannheim, Germany) and Professor G. Fey, University of Erlangen (Erlangen, Germany). Control hybridizations were performed with a 1 kb *Pst*I fragment of a β-actin cDNA (kindly provided by Dr. E. Arzt, University of Buenos Aires, Bueos Aires, Argentina). The inserts were prepared and labeled with the multiprime labeling method in accordance with the manufacturer's instruction (Boehringer-Mannheim). Nylon membranes (Amersham, Braunschweig, Germany) were prehybridized for 4 h at 42°C in a solution containing 50% formamide, 5 × SSPE (standard saline phosphate etyhenediaminetetraacetic acid; 1 × SSPE contains 0.15 M NaCl, 10 mM NaH_2PO_4, 1.25 mM EDTA), sheared denatured salmon sperm DNA (0.1 mg/ml), 5 × Denhardt's solution and 0.1% sodium dodecyl sulfate (SDS), pH 7.4. The filters were then hybridized for 15–20 h at 42°C in the same solution, containing approximately 2×10^6 dpm/ml ^{32}P-labeled cDNA probe. After hybridization, filters were washed twice for 10 min at room temperature in 2 × SSC (standard saline citrate; 1 × SSC contains 0.15 M NaCl, 0.03 M sodium citrate), 0.1% SDS, once at 50°C in 2 × SSC, 0.1% SDS and once at 50°C in 0.2 × SSC, 0.1% SDS. Subsequently, the filters were autoradiographed. The size of the mRNA was estimated by comparison with 18S and 28S rRNA. The cDNA was stripped off and a second hybridization was performed with the actin probe.

2.6. Corticosterone determinations

Vehicle and oligodeoxynucleotides were injected i.c.v. at 08.00 h, using a Hamilton syringe connected to a 30 gauge injection needle via poly-ethylene tubing, as described in Section 2.2. Blood samples (300 μl) were withdrawn before the injections and at distinct intervals after the injections and collected in ice-chilled EDTA-coated tubes containing 10 μl aprotinin. Plasma was prepared, and the samples were assayed for corticosterone using a commercial corticosterone radioimmunoassay (ICN Biochemicals, Meckenheim, Germany). The interassay coefficient of variation for corticosterone was about 3%, the

intraassay coefficent was about 4.5% and the detection limit was 1.5 ng/ml.

2.7. Statistics

Results are expressed as means ± S.E.M. For statistical evaluation, the values of each treatment group were submitted to a two-way analysis of variance (ANOVA) with repeated measures design (treatment as between subjects factor and time as within subjects factor). Tukey's test was performed for post-hoc comparisons to determine when and where significant interaction factor effects occurred. The relationship between dose and area under the curve (AUC) of body temperature was tested with polynomial contrasts in ANOVA. For post-hoc comparisons an adjusted level of significance was used (Bonferroni correction) to keep the type I error below 0.05.

3. Results

3.1. Phosphorothioate oligodeoxynucleotides dose-dependently induce fever and sickness behavior independent of the nucleotide sequence

First we studied the effect of four i.c.v.-injected phosphorothioate oligodeoxynucleotides (missense oligodeoxynucleotide, arginine vasopressin sense, oxytocin antisense and oxytocin sense oligodeoxynucleotides) on body temperature, locomotor activity, food intake and water consumption over a 24 h period. In addition four different doses (1, 5, 25 and 50 μg; i.c.v.) of arginine vasopressin antisense oligodeoxynucleotide were injected in order to study the dose–response profile on the parameter described above. We selected a dose of 50 μg or less of the oligodeoxynucleotides because a previous study had shown that i.c.v. injections of this amount of the arginine vasopressin antisense oligodeoxynucleotide optimally inhibited the expression and activity of arginine vasopressin (Skutella et al., 1994).

The time-course of body temperature in 1 h intervals after the injection of various oligodeoxynucleotides is depicted in Figs. 1 and 2. It should be noted that for comparison the same vehicle control group was used in all experiments. Previous experiments yielded that vehicle controls are stable over time. After the injection of each of the five different phosphorothioate oligodeoxynucleotides body temperature gradually increased. Maximal values (38.9 ± 0.2 to 39.4 ± 0.2 versus 37.5 ± 0.2 in controls) occurred between 4 and 13 h post-injection. After the peak, body temperature declined, but it was still elevated compared to the controls at the end of the 24 h recording period. There were no significant differences between the various phosphorothioate oligodeoxynucleotides in their effect on body temperature. Thus, body temperature was elevated independent of the nucleotide sequence.

Administration of arginine vasopressin antisense oligodeoxynucleotide induced a dose-dependent increase

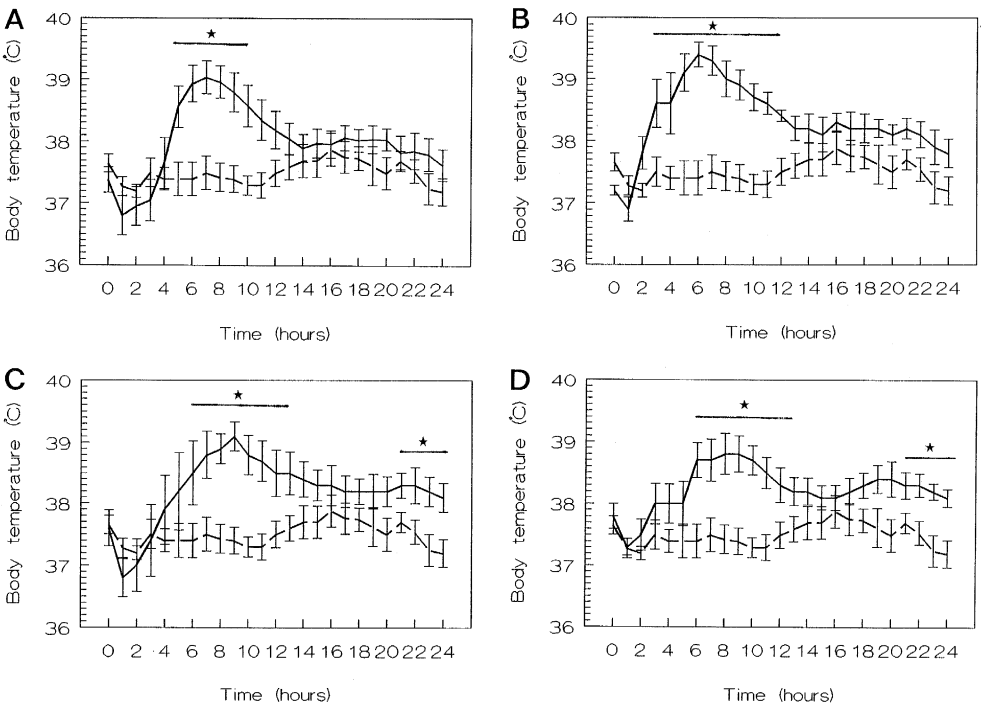

Fig. 1. Body temperature in rats after single i.c.v. injections of phosphorothioate oligodeoxynucleotides with different nucleotide sequences. The animals ($n = 7$–12 per group) were injected with vehicle (dashed lines) or 50 μg missense oligodeoxynucleotide (A) or arginine vasopressin sense (B), oxytocin antisense (C) or oxytocin sense (D) oligodeoxynucleotide (solid lines). Body temperature was recorded over a 24 h period using radiotelemetry; mean values ± S.E.M. for 1 h intervals are given. * $P < 0.05$ versus vehicle.

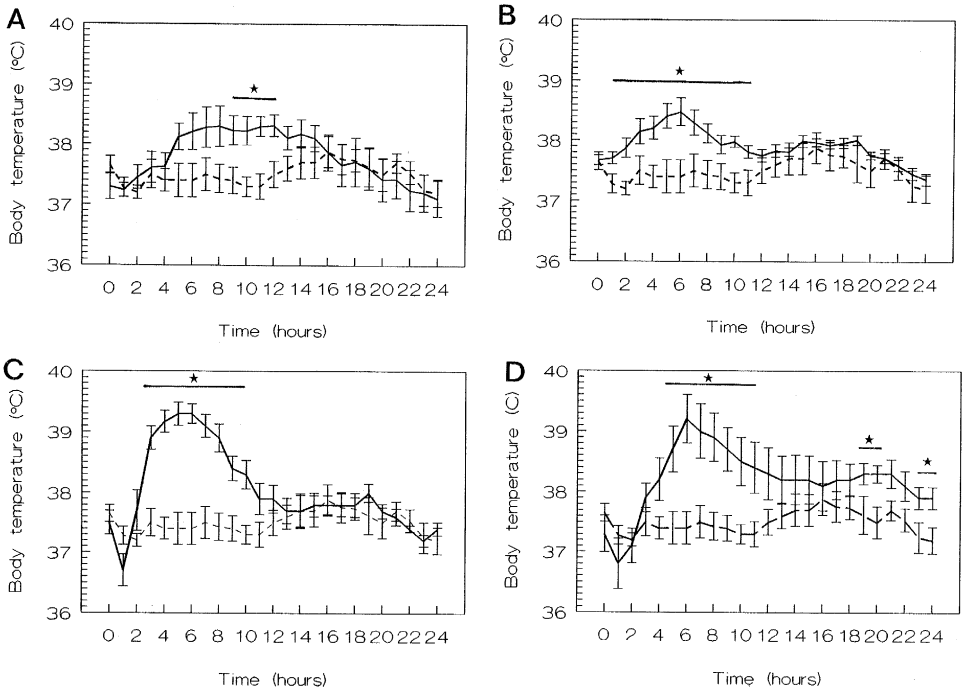

Fig. 2. Body temperature in rats after i.c.v. injections of 1–50 μg of an arginine vasopressin antisense phosphorothioate oligodeoxynucleotide. The animals ($n = 7$–12 per group) were injected with vehicle (dashed lines; it should be noted that for comparison the same vehicle control group was used as in the experiments depicted in Fig. 1 or 1 μg (A), 5 μg (B), 25 μg (C) or 50 μg (D) oligodeoxynucleotide (solid lines). Body temperature was recorded over a 24 h period using radiotelemetry; mean values ± S.E.M. for 1 h intervals are given. * $P < 0.05$ versus vehicle.

in body temperature. The fever response to 1 and 5 μg of the oligodeoxynucleotide was milder than to doses of 25 and 50 μg (effect of time: $F(24,768) = 16.88$, $P < 0.0001$; interaction time × treatment: $F(96,768) = 3.33$, $P < 0.0001$). One-way ANOVA with polynomial contrasts revealed a linear trend between the dose of the oligodeoxynucleotide (1–50 μg) and the AUC of body temperature during the interval 2–12 h after the injection (effect of dose: $F(4,32) = 4.92$, $P < 0.01$; test of linear contrast $P < 0.001$).

Treatments with the different oligodeoxynucleotides significantly reduced the locomotor activity of the animals in comparison with the controls irrespective of the nucleotide sequence (Fig. 3A). Due to the circadian rhythm of the rat, we observed a low level of spontaneous activity in vehicle-treated animals before lights were switched off (i.e., for the first 9 h after the injections). During this period, treatment with oligodeoxynucleotides had no influence on locomotor activity. However, at nighttime (between 9 and 21 h after the injections), the locomotor activity of the controls increased, whereas the activity of the animals treated with oligodeoxynucleotides remained in the low range of the activity values that were found in controls during the light phase.

Administration of various doses of arginine vasopressin antisense oligodeoxynucleotide had also a dose-dependent effect on locomotor activity (Fig. 3B). Thus, locomotor activity of both controls and arginine vasopressin antisense oligodeoxynucleotide-treated animals increased at night, but the increase was significantly less in all oligodeoxynucleotide-treated animals than in controls (effects of treatment: $F(7,189) = 13.7$, $P < 0.001$; interaction time × treatment: $F(21,189) = 2.15$, $P < 0.001$) The suppressive effect of the oligodeoxynucleotide was maximal at 50 μg and minimal at 1 μg, i.e., at the lowest dose the inhibition of locomotor activity was observed only for one time interval.

Although animals treated with different oligodeoxynucleotides appeared to consume less food than vehicle-treated controls (Fig. 4A), the difference was not statistically significant. Food intake was also not affected by 1 and 5 μg of arginine vasopressin antisense oligodeoxynucleotide but appeared to be inhibited by the higher doses although the decrease was not significant (Fig. 4B). Water intake was not significantly affected by injection of oligodeoxynucleotides during the first 12 h after the treatment, whereas during the second 12 h it was significantly lower in the oligodeoxynucleotide-treated animals than in the controls (interaction time × treatment: $F(5,37) = 3.765$, significance of $P < 0.05$) (Fig. 5A). arginine vasopressin antisense, in contrast to the other oligodeoxynucleotides, increased water intake 2- to 3-fold during the first 12 h after the injections (Fig. 5B). However, the effect failed to reach significance.

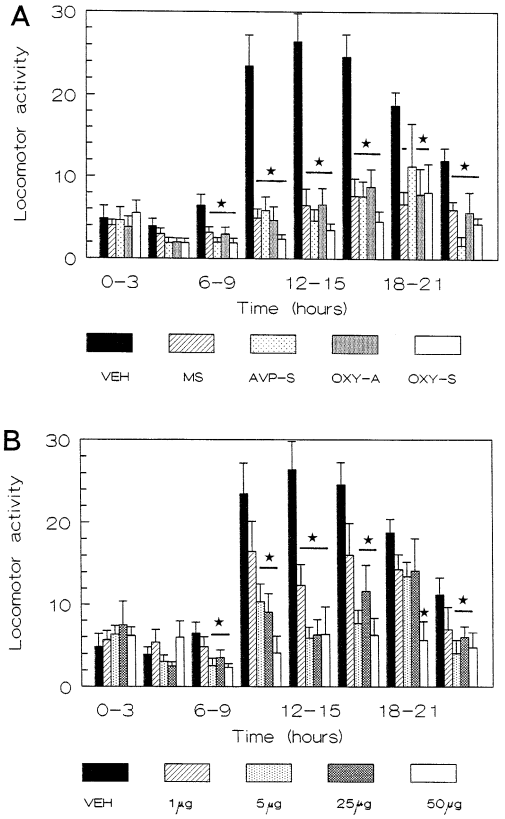

Fig. 3. Locomotor activity in rats after single i.c.v. injections of phosphorothioate oligodeoxynucleotides with different nucleotide sequences. The animals ($n = 7–12$ per group) were injected with vehicle (Veh) or 50 μg missense, arginine vasopressin sense, oxytocin antisense or oxytocin sense oligodeoxynucleotides (A) or with increasing doses of arginine vasopressin antisense oligodeoxynucleotide (1, 5, 25 and 50 μg) (B). Locomotor activity was recorded over a 24 h period using radiotelemetry; mean values ± S.E.M. for 3 h intervals are given. * $P < 0.05$ versus vehicle.

3.2. Oligodeoxynucleotides induce fever and sickness behavior independent of the chemical modification

In the next experiment we compared the effect of an missense phosphorothioate oligodeoxynucleotide, an missense oligodeoxynucleotide with 3′-3′-inverted internucleotidic linkage and native double-stranded salmon sperm DNA on body temperature (Fig. 6), locomotor activity, food and water intake (Fig. 7). In comparison with the oligodeoxynucleotides, 50 μg salmon sperm DNA resulted in a more rapid onset of fever and the fever ended 6 h before that caused by the oligodeoxynucleotides (factor treatment: $F(2,15) = 13.217$, $P < 0.0001$; factor time $F(24,590) = 12.199$, $P < 0.001$; interaction of both factors: $F(48,390) = 3.934$, $P < 0.0001$).

The two chemically different oligodeoxynucleotides suppressed locomotor activity (Fig. 7A) to the same extent, whereas native double-stranded DNA was less potent, i.e., locomotor activity was inhibited during fewer time intervals (factor treatment: $F(3,29) = 12.751$, $P < 0.0001$; factor time: $F(7,203) = 20.053$; $P < 0.0001$, interaction time × treatment: $F(21,203) = 7.547$, $P < 0.001$). The oligodeoxynucleotides suppressed food intake (factor treatment: $F(3,34) = 4.234$, $P < 0.01$, factor time: $F(2,34) = 166.452$, $P < 0.0001$), whereas salmon sperm DNA (Fig. 7B) had only a non-significant anorectic effect. The end-inverted oligodeoxynucleotide inhibited water intake during the first 12 h after the injection compared to controls and was more potent than the phosphorothioate oligodeoxynucleotide during the second 12 h interval after the treatment (Fig. 7C) (factor treatment: $F(3,28) = 4.551$, $P < 0.01$; factor time $F(1,28) = 16.615$, $P < 0.0001$; interaction time × treatment: $F(3,28) = 3.066$, $P < 0.05$). However, the differences in drinking rate between the two oligodeoxynucleotides were not significant.

3.3. Fever and sickness behavior in response to oligodeoxynucleotides are neither due to by-products of the chemical synthesis nor caused by endotoxin contamination

To exclude the possibility that by-products of the chemical synthesis caused fever and sickness behavior, we

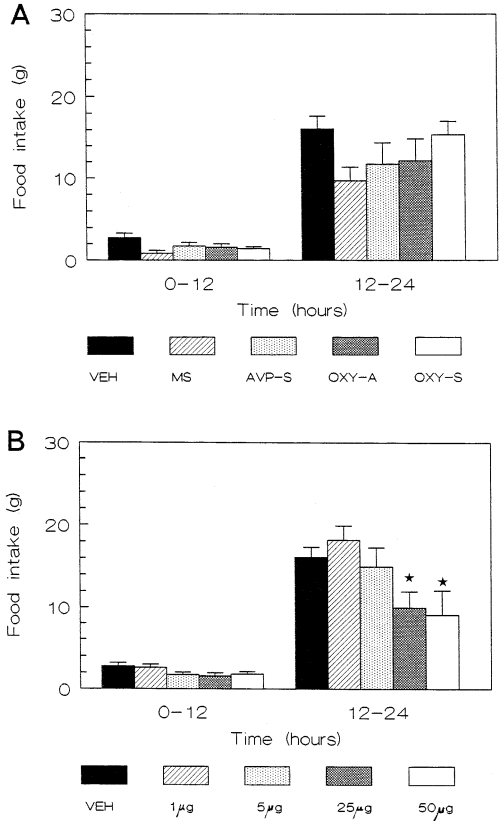

Fig. 4. Food intake in rats after single i.c.v. injections of phosphorothioate oligodeoxynucleotides with different nucleotide sequences. The animals ($n = 7–12$ per group) were injected with vehicle (Veh) or 50 μg missense, arginine vasopressin sense, oxytocin antisense or oxytocin sense oligodeoxynucleotides (A) or with increasing doses of arginine vasopressin antisense oligodeoxynucleotide (1, 5, 25 and 50 μg) (B). * Food intake was determined by weighing food pellets at 12 h intervals. Mean values ± S.E.M. for 12 h intervals are given.

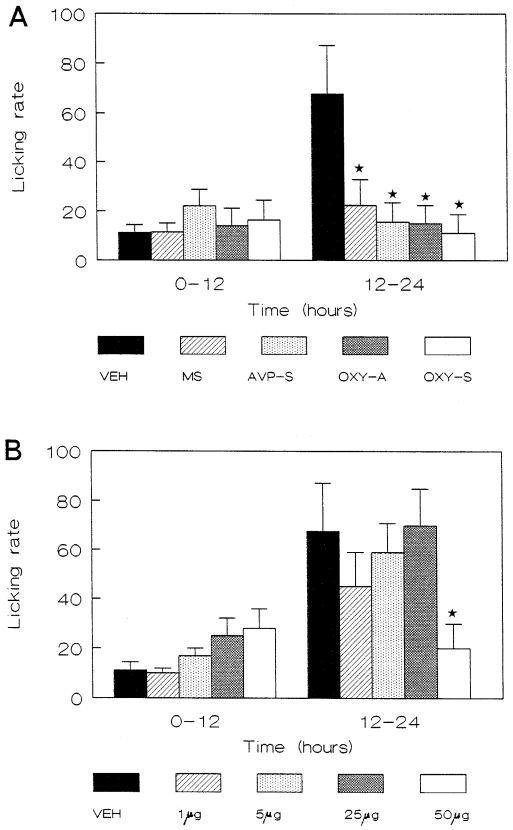

Fig. 5. Water consumption in rats after single i.c.v. injections of phosphorothioate oligodeoxynucleotides with different nucleotide sequences. The animals ($n = 7–12$ per group) were injected with vehicle (Veh) or 50 μg missense, arginine vasopressin sense, oxytocin antisense or oxytocin sense oligodeoxynucleotides (A) or with increasing doses of arginine vasopressin antisense oligodeoxynucleotide (1, 5, 25 and 50 μg) (B). Licking rates were measured with a lick sensor at the drinking nipple. Mean values ± S.E.M. for 12 h intervals are given.

tested the arginine vasopressin antisense oligodeoxynucleotide (50 μg, i.c.v.) after it had been subjected to an additional purification step by ion exchange chromatography. The purification reduced neither the fever response to the oligodeoxynucleotide (Fig. 8A, solid line) nor the induction of sickness behavior (data not shown) compared with substances that had been purified by the suppliers only.

Furthermore, a mock synthesis was carried out to obtain putative by-products from the chemical synthesis. Injection of mock-synthesized material was accompanied neither by fever (Fig. 8A, dashed line) nor by sickness behavior (data not shown).

To exclude the possibility that the fever response to oligodeoxynucleotides was caused by contamination with endotoxin, we measured the concentration of endotoxin in all samples containing oligodeoxynucleotides and native DNA. For this purpose we used a colorimetric assay that is based on the *Limulus* assay. The endotoxin content (expressed as standard *E. coli* 0111:B4 lipopolysaccharide) in 5 μl of the injection solutions, containing 50 μg of the oligodeoxynucleotide, ranged from 1 pg (missense-inverted) to 1.4 pg (oxytocin sense). Salmon sperm DNA (50 μg in a total volume of 5 μl) contained 2.2 pg endotoxin. When we combined 26.7 pg/ml endotoxin from the sample containing the arginine vasopressin antisense oligodeoxynucleotide with 25.0 pg/ml standard endotoxin, we found 50.2 pg/ml endotoxin in the mixture. Therefore, we conclude that oligodeoxynucleotides do not interfere with the endotoxin assay.

Administration of 50 pg *E. coli* standard endotoxin (i.c.v.) was not sufficient to evoke a fever response (Fig. 8B, dashed line). In contrast, 25 μg arginine vasopressin

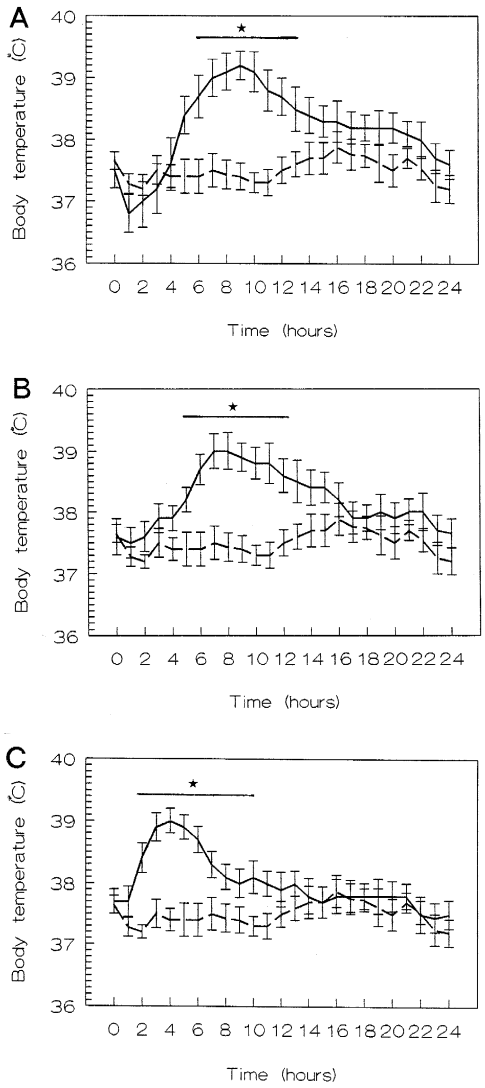

Fig. 6. Body temperature in rats after a single i.c.v. injection of an missense phosphorothioate oligodeoxynucleotide (A), an missense end-inverted oligodeoxynucleotide (B) or double-stranded salmon sperm DNA (C). The animals ($n = 7–12$ per group) were injected with vehicle (dashed lines; it should be noted that for comparison the same vehicle control group was used as in the experiments depicted in Fig. 1) or 50 μg of one of the nucleic acids (solid lines). Body temperature was recorded over a 24 h period using radiotelemetry; mean values ± S.E.M. for 1 h intervals are given. * $P < 0.05$ versus vehicle.

antisense oligodeoxynucleotide, containing 0.7 pg endotoxin, increased body temperature (Fig. 8B, solid line). Preincubation of arginine vasopressin antisense oligodeoxynucleotide (final concentration 5 mg/ml) with polymyxin (final concentration 2 mg/ml) for 30 min at 37°C did not block the fever induction by the oligodeoxynucleotide (Fig. 8C, solid line), whereas polymyxin alone, injected i.c.v. at the same dose (i.e., 20 μg), had no effect on body temperature (Fig. 8C, dashed line).

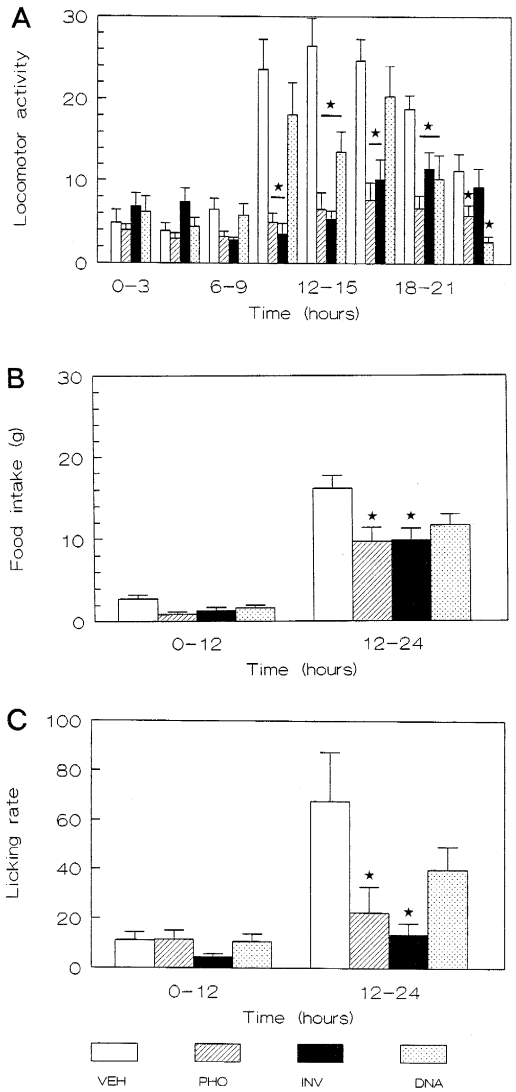

Fig. 7. Locomotor activity (A), food intake (B) and licking rate (C) in rats after a single i.c.v. injection of missense phosphorothioate oligodeoxynucleotides (PHO), missense end-inverted oligodeoxy-nucleotides or double-stranded salmon sperm DNA (DNA). The animals ($n = 7–12$ per group) were injected i.c.v. with vehicle or 50 μg of one of the nucleic acids. Locomotor activity was recorded over a 24 h period using radiotelemetry; values ± S.E.M. for 3 h intervals are given; food intake was determined by weighing food pellets at 12 h intervals and licking rate was counted with a lick sensor at the drinking nipple; mean values ± S.E.M. for 12 h intervals are given. * $P < 0.05$ versus vehicle.

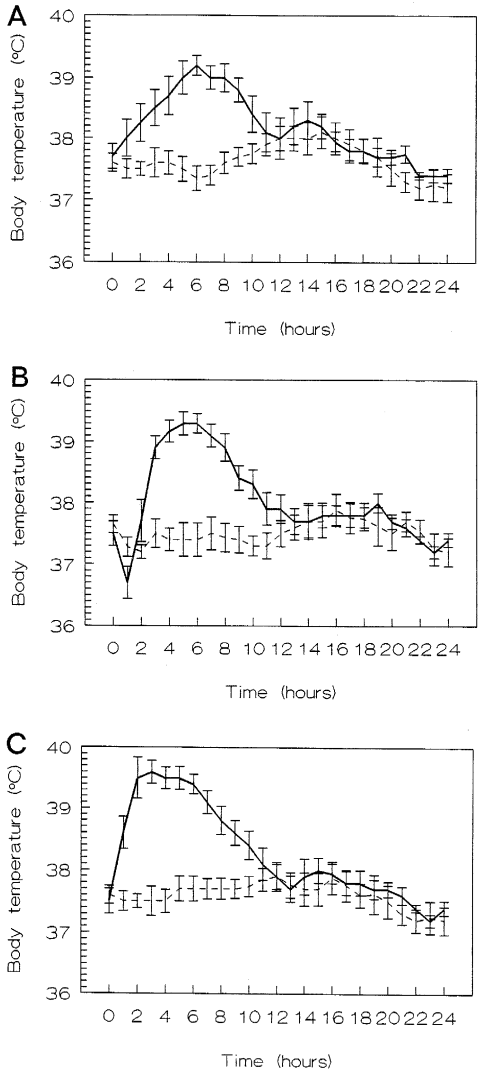

Fig. 8. Body temperature in rats treated with putative contaminants of oligodeoxynucleotides and highly purified arginine vasopressin antisense oligodeoxynucleotide. The animals ($n = 6–10$ per group) were injected i.c.v. with mock-synthesized arginine vasopressin antisense oligodeoxynucleotide (A, dashed line), 50 μg arginine vasopressin antisense oligodeoxynucleotide after further purification by ion exchange chromatography (A, solid line), 50 pg *E. coli* standard endotoxin (B, dashed line), 25 μg arginine vasopressin antisense oligodeoxynucleotide (B, solid line), 20 μg polymyxin (C, dashed line) or 25 μg arginine vasopressin antisense oligodeoxynucleotide (final concentration 5 mg/ml) preincubated with polymyxin for 30 min at 37°C (final concentration 2 mg/ml) (C, solid line). Body temperature was recorded over a 24 h period using radiotelemetry; mean values ± S.E.M. for 1 h intervals are given.

3.4. Centrally administered phosphorothioate oligodeoxynucleotides cause a central and a peripheral induction of interleukin 6 mRNA

To determine whether the increase in body temperature after i.c.v. injection of oligodeoxynucleotide is accompanied by synthesis of pyrogenic cytokines, we determined

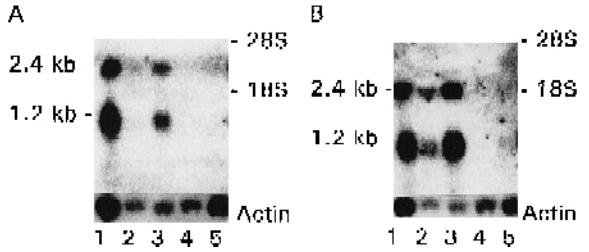

Fig. 9. Northern blot analysis of interleukin 6 mRNA in brain (A) and spleen (B) of rats treated with oligodeoxynucleotides. 3 h (lanes 2 and 3) and 24 h (lanes 4 and 5) h after i.c.v. injection of vehicle (lanes 2 and 4) or 50 μg missense phosphorothioate oligodeoxynucleotide (lanes 3 and 5), the animals were decapitated, and RNA was isolated and analyzed as described in the Section 2. As a positive control (lane 1) interleukin 6 mRNA from the spleen of lipopolysaccharide-treated animals (i.p., 100 μg/kg, 3 h post-injection) is shown.

interleukin 6 mRNA in brain and spleen by Northern blot analysis. As a positive control we used RNA from the spleen of animals 3 h after i.p. injection of lipopolysaccharide (100 μg/kg). 3 h after i.c.v. administration of the missense phosphorothioate oligodeoxynucleotide (50 μg), interleukin 6 mRNA was induced in brain and spleen (Fig. 9). The elevated interleukin 6 mRNA content in both tissues was transient and not detectable 24 h after the treatments.

3.5. Missense oligodeoxynucleotides elevate circulating corticosterone

Finally, we determined whether the stress due to inflammation and fever after i.c.v. injection of oligodeoxynucleotides is accompanied by elevated concentrations of plasma corticosterone. As shown in Fig. 10, administration of 50 μg missense phosphorothioate oligodeoxynucleotide increased the plasma corticosterone concentration for at least 4 h (factor treatment: $F(1,14) = 13.344$, significance of $P < 0.01$; factor time: $F(4,56) = 10.962$, significance of $P < 0.0001$; interaction time × treatment: $F(4,56) = 3.870$, significance of $P < 0.01$).

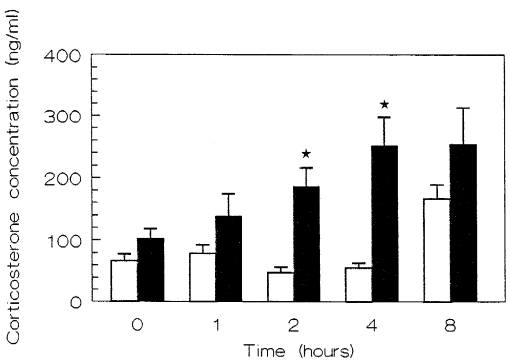

Fig. 10. Plasma corticosterone in rats after a single i.c.v. injection of oligodeoxynucleotides. At 08.00 h the animals ($n = 8–12$ per group) were injected with vehicle (open bars) or 50 μg of a phosphorothioate missense oligodeoxynucleotide (black bars). Blood samples were withdrawn before the injections and at the intervals shown after the injections. Plasma was prepared and the samples were assayed for corticosterone. * $P < 0.05$ versus vehicle.

4. Discussion

Side effects of oligodeoxynucleotides are not very well documented despite their wide use in experimental pharmacology and physiology. A few studies have addressed sequence-independent effects of oligodeoxynucleotides (Perez et al., 1994; Galbraith et al., 1994) and in vivo toxicity of systemically administered oligodeoxynucleotides (Sarmiento et al., 1994). We report here that phosphorothioate and end-inverted oligodeoxynucleotides elevate body temperature, suppress food and fluid intake and inhibit locomotor activity after a single injection into the lateral ventricle of the rat brain. We tested five different nucleotide sequences, all of which produced similar changes in the autonomous and behavioral parameters. In addition we observed fever after injection of native double-stranded DNA, suggesting that the nucleic acid structure of oligodeoxynucleotides underlies the effects. The finding that the fever response to native DNA was shorter than that to oligodeoxynucleotides may reflect increased stability of chemically modified oligodeoxy-nucleotides.

We used commercially available oligodeoxynucleotides, which had been purified by the suppliers. Additional purification of oligodeoxynucleotides by ion exchange chromatography did not reduce the side effects, which strongly indicates that either nucleic acids or their metabolites, but not by-products of the chemical synthesis, caused the autonomous and behavioral effects we measured. In an additional experiment we showed that putative by-products, which had been synthesized by a mock oligodeoxynucleotide synthesis, are biologically ineffective. Moreover, native salmon sperm DNA, which was not chemically synthesized, produced fever and sickness behavior.

Furthermore, we can exclude the possibility of bacterial endotoxin, which is ubiquitously present and contaminates synthetic and biological fluids (Homma et al., 1984), of having caused the fever and sickness-like behavior, as the endotoxin content of the oligodeoxynucleotides was not pyrogenic. Moreover, polymyxin, which binds and inactivates endotoxins (Morrison and Curry, 1979), did not inhibit fever in response to oligodeoxynucleotides.

Several mechanisms may account for the induction of fever, reduced locomotor and diminished feeding behavior by nucleic acids in the central nervous system. For example, oligodeoxynucleotides may exhibit cytotoxic properties in the brain similar to their effects in cell culture (Crooke, 1991). Cytotoxicity may be caused directly by modified or unmodified nucleic acids or both or indirectly by degradation products, i.e., nucleosides or nucleoside

derivatives. Limited tissue damage may be associated with generation of inflammatory mediators, including eicosanoids and cytokines (Shimizu and Wolfe, 1990; Schöbitz et al., 1994b). It is known that the brain is capable of producing large quantities of inflammatory mediators despite a generally weak inflammatory response (Perry and Andersson, 1992). These autacoids can rapidly trigger a systemic acute-phase response, elevate body temperature and can produce behavioral depression (Schöbitz et al., 1994a) as observed in our study. In particular serum and tissue content of the pyrogenic cytokine interleukin 6 correlates with the size of injuries (Nijsten et al., 1987; Di Padova et al., 1991), the degree of fever (Nijsten et al., 1987) and the severity of sepsis (Damas et al., 1991) and therefore the interleukin 6 content of a given tissue is an accurate measure of the extent of the inflammatory response. The brain of healthy rats expresses small quantities of interleukin 6 (Schöbitz et al., 1993), whereas after brain damage or i.c.v. injection of lipopolysaccharide (Muramami et al., 1993; Schöbitz et al., 1994b) the amount of interleukin 6 is drastically increased. In the present study centrally administered phosphorothioate oligodeoxynucleotides caused an induction of interleulin 6 mRNA in the brain and in the spleen as shown by Northern blot analysis suggesting also an increase in the interleukin 6 content in these tissues.

The cellular uptake of oligodeoxynucleotides is thought to be accomplished by a number of different mechanisms including receptor-mediated endocytosis (Akhtar and Juliano, 1992). The interaction of nucleic acids with the cell membrane may be accompanied by activation of membrane-bound enzymes, including phospholipase A_2, thereby triggering the formation of pyrogenic prostaglandins (Smith, 1989). Cell surface receptors that bind oligodeoxynucleotides with a dissociation constant below 1 μM have been described by Yakubov et al. (1989). The observation that double-stranded DNA can also associate with these receptors is in line with our finding that oligodeoxynucleotides and native DNA produce largely similar effects after central administration. Lymphocytes also express a 30 kDa binding protein for double-stranded DNA (Bennett et al., 1985). It is thus conceivable that eukaryotic cells contain several binding sites for nucleic acids, which might have the function of triggering inflammatory processes to combat pathogenic microorganisms efficiently, as is thought to be the case for lipopolysaccharide and muramyl peptide receptors (Lynn and Golenbock, 1992). Accordingly, binding of extracellular nucleic acids to the corresponding receptors might signal the presence of microorganisms, viruses and cell debris. It should be noted that nucleic acids, particularly polyI:C, have been known for some time to produce fever in experimental animals (Lindsay et al., 1969).

The results of our study indicate that centrally administered oligodeoxynucleotides produce transient, sequence-independent effects. Although these side effects can but do not necessarily interfere with the intended sequence-specific effect (Schöbitz et al. submitted), the behavioral depression after i.c.v. administration of oligodeoxynucleotides should be taken into account when behavioral paradigms are studied which are affected by a sickness-like behavior as observed in the present study.

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