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Treatment with a polyunsaturated fatty acid prevents deleterious effects of Ro4-1284

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Abstract

Ro4-1284 (2-Ethyl-1,3,4,6,7,llb-hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzo[*a*] quinolizin-2-ol hydrochloride), a benzoquinolizine, is a potent dopamine depletion agent whose acute and chronic administration results in a (1) deterioration of learning in the Morris Water Maze and passive avoidance tasks, (2) decrease in locomotion and rearing, (3) intense hypothermia, and (4) decrease in the percentage of polyunsaturated fatty acids and an increase in the level of cholesterol in neuronal membranes. Pretreatment with a specific mixture of free polyunsaturated fatty acids prevents most of the behavioral, physiological, and biochemical effects of Ro4-1284 except for rearing. We propose that the dopamine-mediated functions tested in this study are dependent on the interaction of intact dopamine D_1 and D_2 receptors. Rearing, which is controlled only by dopamine D_1 receptors, remained, therefore, unaffected. Our hypothesis is that SR-3 exerts its beneficial effects by normalizing the structure and function of the neuronal membrane and by restoring dopamine D_2 receptor functions. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ro4-1284; Morris Water Maze; Passive avoidance; Hypothermia; Dopamine; Linolenic acid; α-Linolenic acid

1. Introduction

Previous studies indicate that free fatty acids may, under certain conditions, exert a strong influence on neuronal activity. Polyunsaturated fatty acids are more active in the central nervous system than other groups of fatty acids. Polyunsaturated fatty acids may act at the level of membrane function by (1) altering 'membrane fluidity', where polyunsaturated fatty acids may 'fluidize' the membrane and cholesterol may 'harden' the membrane, (2) interacting with cellular and membrane-bound enzymes, (3) acting on ionic channels, and (4) modifying neurotransmitter and peptide receptors (Yehuda et al., 1997a,b).

The relationship between the dopaminergic system and the profile of free polyunsaturated fatty acids in the brain has been reported in a variety of studies and experimental protocols. In α-linolenic (18:3 $n-3$) acid deficient rats, the number of dopamine receptors in the frontal cortex is decreased (Canonico, 1989; Delion et al., 1996). Polyunsaturated fatty acids deficiency leads to a modification of the internalization of dopamine receptor into the storage pool (Zimmer et al., 1998). It seems that polyunsaturated fatty acids deficiency has a particularly damaging effect on the dopamine receptor system. Rats with long term $n-3$ deficiency develop a selective decrease in the level of dopamine and dopamine and in dopamine D_2 receptor binding, and an increase in the serotonin receptor density in the frontal cortex. However, the levels of serotonin and norepinephrine are not affected (Reisbick and Neuringer, 1997). Other studies found that the release of dopamine increases the level of arachidonic acid [20:4 ($n-6$)] (Kessler and Yehuda, 1985; Vaidyanathan et al., 1994; Kerttula et al., 1995a,b). In turn, increased levels of arachidonic acid cause the release of dopamine from brain neurons (Dunwiddie et al., 1990), which serves to inhibit lipid peroxidation (Miura et al., 1996), just as cyanide treatment inhibits dopamine release and stimulates lipid peroxidation (Kaplan et al., 1994; Lafuente et al., 1994; Karanth et al., 1995), and the number of dopamine D_2 receptors is increased by docosahexaenoic acid (22:6 $n-3$) (Trampus and Ongini, 1990; Senaris et al., 1993; Rego and Oliveira, 1995; Makita et al., 1996).

Functionally, the dopaminergic system mediates several types of behavior, and significant changes are manifest in

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polyunsaturated fatty acid deficient rats. In addition, the dopaminergic system is also involved in several types of learning tasks such as the Morris Water Maze. Although there is no agreement on the exact neuroanatomical and neurochemical basis for the behavioral changes, it is known that navigation in the Morris Water Maze task is dependent on the integrity of the dopaminergic mesohippocampal connection (Gasbarri et al., 1996) and the mesolimbic and nucleus accumbens dopaminergic system (Ploeger et al., 1994). Other studies have shown that dopaminergic in the caudate is also involved in mediating learning (Lee et al., 1994). Similarly, three dopamine receptors types [D_1, D_2 and D_3] (Hersi et al., 1995; Sigala et al., 1997) have been implicated in Morris Water Maze learning. It is not surprising therefore that a decrease in $n-3$ and $n-6$ levels in the brain results in impaired Morris Water Maze learning (Coscina, 1997).

In passive avoidance learning designs, increased levels of $n-3$ and $n-6$ fatty acids in the brain are correlated with improved passive avoidance learning (Sato et al., 1995; Minami et al., 1997). The level of arachidonic acid increases during the passive avoidance training period (Clements and Rose, 1996) while lesions in the septum (an area rich in dopamine D_3 receptors) impair passive avoidance learning (Riekkinen et al., 1996). The role of dopamine in thermoregulation is well known (Salmi, 1998).

Recently, we demonstrated that a mixture of free essential fatty acids [linoleic (18:2 $n-6$) and α-linolenic (18:3 $n-3$)] is able to increase the level of polyunsaturated fatty acids and to reduce the levels of cholesterol in brain neuronal membranes (Yehuda et al., 1996; Yehuda et al., 1998). This mixture (which we have called SR-3) improves learning in normal rats, as assessed by Morris Water Maze and other tasks (Yehuda and Carasso, 1993). It is also able to reverse learning deficits induced by the neurotoxins AF64A and 5,7-dihydroxytryptamine (Yehuda et al., 1995) or by brain iron deficiency (Yehuda et al., 1994). SR-3 has been shown to elevate the pain threshold, improve thermoregulation (Yehuda and Carasso, 1993), provide protection against seizure induced by various treatments (Yehuda et al., 1994), and improve symptoms in experimental models of multiple sclerosis (Experimental Allergic Encephalomyelitis), such as reversing the decrease in polyunsaturated fatty acid levels, the increase in cholesterol levels, and the impairment of Morris Water Maze and passive avoidance learning (Yehuda et al., 1997c). The mediation by DA of the effects of polyunsaturated fatty acids or SR-3 on dopaminergic functions has not been studied. A preliminary study showed that chronic administration of SR-3 does not modify the level of DA in the striatum. However, pre-treatment with SR-3 prevents the decrease in DA level induced by Ro4-1284 (see Section 4).

The compound Ro4-1284 (2-Ethyl-1,3,4,6,7,llb-hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzo[*a*] quinolizin-2-ol hydrochloride), (Hoffman-LaRoche, Basel) has been established as a potent DA-depleting agent (Da Prada, 1977; German et al., 1981; Fuller and Hemrick-Luecke, 1985; Colzi et al., 1992) and provides an animal model of benign essential blepharospasm. Burkard et al. (1989) demonstrated that challenging rats with a dose of 20 mg/kg Ro4-1284 was sufficient to induce blepharospasms, a finding that we were able to replicate and that we report elsewhere. The primary aims of this study were to evaluate the effectiveness of SR-3 to reverse the biochemical and behavioral changes that are due to the depletion of DA by Ro4-1284, including deficits in learning and thermoregulation, and alterations in the fatty acid profile and cholesterol level of neuronal membranes.

2. Materials and methods

2.1. Test materials

α-Linolenic (0.92 g/ml) and linoleic (0.90 g/ml) free fatty acids, both 99% pure as evaluated by capillary gas chromatography (Sigma, St. Louis, MO, USA (L2367 and L1376) were stored in the dark at 4°C. A fresh stock solution (1 ml) was prepared every 3 days by mixing 0.40 ml of α-linolenic and linoleic acid in ratio of 1:4, mineral oil (0.59 ml) and α-tocopherol (0.02 ml). Ro4-1284 was dissolved in saline (0.9 NaCl). The treatment dose was always 40 mg/kg.

2.2. Animals

A total of 72 Sprague–Dawley rats (six independent groups of $n=12$) were used in the study. The rats were housed individually in hanging, stainless steel, wire-mesh cages in a well-ventilated and air-conditioned room maintained at an average of 22°C and a relative humidity of about 45%. The room was illuminated by fluorescent light that simulated the spectrum of the sun (Vita-Lite; Dura-Test; Clifton, NJ) to permit an artificial 24-h cycle of 12-h of light daily (from 6 a.m. to 6 p.m.). Tap water and Altromine C-1000 diet were available ad libitum.

2.3. Experimental design

Treatment conditions lasting 3 weeks were as follows:

1. no treatment control;
2. saline control;
3. saline control followed by a single dose of Ro4-1284 on day 22;
4. saline control and 10 days of Ro4-1284 beginning on day 22;
5. SR-3 followed by single dose of Ro4-1284 on day 22;
6. SR-3 followed by 10 days of Ro4-1284 beginning on day 22. Animals receiving SR-3 were given daily injections of 40 mg/kg.

2.4. Synaptosomes and determination of fatty acids and cholesterol

After the rats were killed by rapid decapitation (to avoid interference with fatty acids by use of anesthetics), the brain was exposed and tissue was collected. The level of fatty acids and cholesterol was determined by gas-chromatography. Synaptosomes were prepared as suggested by Whittaker and Barker (1972). Brain tissues were homogenized on ice in 0.32 M sucrose, pH 7.0, and centrifuged at $23{,}000 \times g$ for 20 min at 1°C. The supernatant was then discarded, and the pellet was resuspended in 6 ml of 0.32 M sucrose, applied to a discontinuous sucrose gradient (0.32 M. 0.8 M., and 1.2 M) and centrifuged at $100{,}000 \times g$ (Model L8-55, Beckman) for 60 min at 1°C. Synaptosomes were then removed from the 0.8–1.2 M sucrose interface with Pasteur pipettes, diluted 1:1 with distilled water and centrifuged at $23{,}000 \times g$ for 20 min at 1°C. The resulting pellet was then resuspended in 1.0 ml of 0.32 M sucrose, rehomogenized and stored at −70°C until analyzed. Lipids were extracted from the membranes in a vial containing 15 ml chloroform/methanol (1:2 vol./vol.) according to Folch-Pi et al. (1957). In previous studies we were able to show that the recovery of synaptosomes was greater than 87% and the purity was determined by electron microscopy. Lipids were analyzed for fatty acid composition by gas chromatography (Varian, SP-2330 Supelco column, BP × 70 Capillary column 50 m 0.33 mm i.d., Model DB-23 SGE) and verified by mass spectrometry (4030 Finnigen-GS-MS, Sunnyvale, CA). Cholesterol was analyzed by gas chromatography, using STIB-5 Supelco capillary columns (15 mm, 0.32 i.d.). Fatty acids were quantified quantification was done by comparison to the GLC standard mixtures GLC30-4-7040, AOCS-4-1019, and GLS60-4-7043 (Supelco, Bellefonte, PA). Data for the following variables were collected: total fatty acids, FA ratio (the ratio of saturated to unsaturated fatty acids) and cholesterol level.

2.5. Measurement of motor activity

The level of motor activity was assessed in an open field apparatus (75 cm × 75 cm) by recording the number of horizontal infrared photobeam crossings. Rearing movements were determined from videotapes made during 15-min sessions.

2.6. Morris Water Maze

The Morris water tank, a circular tank (110 cm in diameter), was filled with water (to the level of 40 cm) which was made opaque by the addition of powdered milk, so that rats swimming in the tank were unable to see the escape platform (7.5 cm in diameter) submerged 2 cm below the surface. Each animal was released facing the wall in one of four predetermined starting points, each separated by 90 cm around the inner perimeter. While in the tank, the rat was able to view surrounding features of the room. Special care was given to keep things in the room at the same location. The rat could navigate in the tank by external cues only. Each rat was tested eight times per day in the tank. The order of the starting point was determined randomly to prevent possible effects of a magnetic field. Each rat was allowed 120 s to find the platform, with an interval of 20 s between trials. The maximum duration of the entire test session for each rat was 16 min. Three rats were tested each hour on each of 3 consecutive days. During this period, the platform was in the same location in the tank. For each of the 24 trials (eight trials on each of 3 days), the latency to reach the platform was recorded. A learning criterion was set that required the rat to reach the platform within 10 s and without any increase in later trials, for at least 19 of the 24 trials.

2.7. Passive avoidance

Passive avoidance was assessed using a box consisting of a bright and a dark compartment. During the training trial (day 20) the rats were placed in the bright compartment. After they entered the dark compartment a shock was delivered (0.5 mA, 3 s). Twenty-four hours later the rats were again placed in the light compartment and the latency to re-enter the dark compartment was measured (maximum duration: 360 s). Short entry latencies indicate poor avoidance learning.

2.8. Body temperature

Body temperature was measured with a telethermometer (YSI Telethermometer, Model 43TA, Yellow Spring, OH).

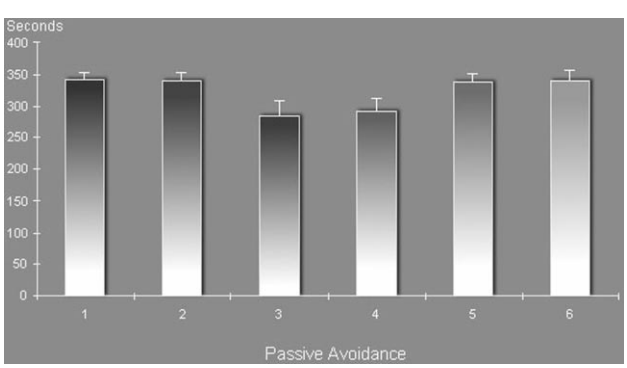

Fig. 1. Effects of Ro4-1284 and SR-3 on performance (measured as latency in seconds) in the passive avoidance test. Data are expressed as means ± S.D. Experimental groups are identified by numbers (1) no treatment control; (2) saline control; (3) saline control followed by a single dose of Ro4-1284 on Day 22; (4) saline control followed by 10 days of Ro4-1284 beginning on Day 22; (5) SR-3 followed by a single dose of Ro4-1284 on Day 22; (6) SR-3 followed by 10 days of Ro4-1284 beginning on Day 22. Animals receiving SR-3 were given daily injections of 40 mg/kg. See text for significant statistical difference.

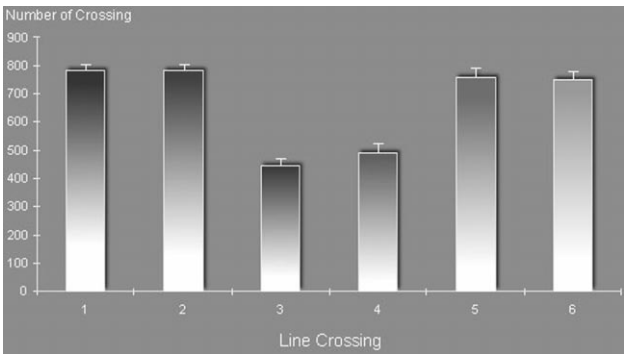

Fig. 2. Effects of Ro4-1284 and SR-3 on the number of line crossings. Data are expressed as means ± S.D. Groups are identified by the same numbers as in Fig. 1. See text for significant statistical differences.

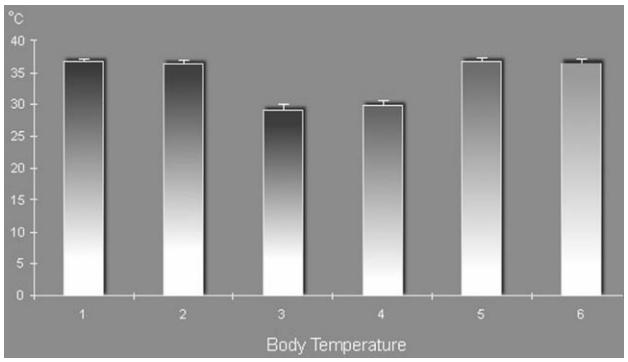

Fig. 4. Effects of Ro4-1284 and SR-3 on body temperature. Data are expressed as means ± S.D. Groups are identified by the same numbers as in Fig. 1. See text for significant statistical differences.

2.9. Statistical analysis

All data are expressed as means and standard deviations (S.D.). The statistical significance of the mean differences was determined by MANOVA followed by one-way analysis of variance (ANOVA). If significant effects were found, means were compared by using Scheffe post hoc comparisons test.

3. Results

3.1. General

MANOVA analysis was performed to determine statistical differences between the experimental groups on PA, line crossing, rearing and thermoregulation variables. MANOVA analysis using Wilks criterion revealed significant results ($F(25,321) = 25.71$, $P < 0.0001$). Significant statistical differences ($P < 0.001$) were found for the following dependent variables: passive avoidance ($F(5,66) = 27.91$), line crossing ($F(5,66) = 359.89$), rearing ($F(5,66) = 111.45$) and body temperature ($F(5,66) = 433.46$. Means and S.D. are presented in Figs. 1–4. Scheffe analysis showed that for each of the independent variables ($\alpha = 0.05$) the Ro4-1284 groups (both chronic and acute) differed from all the other groups. The Ro4-1284 groups did not differ from each other for rearing and line crossing only.

3.2. Passive avoidance

Both acute and chronic Ro4-1284 groups showed impaired PA learning (283.91 and 292.00 s) compared to control rats (340.66). SR-3 treatment reversed the effects of both the acute (339.83) and chronic (338.83) challenge with Ro4-1284 (Fig. 1).

3.3. Line crossing

The general level of motor activity following Ro4-1284 treatments decreased to about 60% of normal motor activity level (784.08) for acute (489.58) and chronic (445.41) groups. Acute and chronic Ro4-1284 groups differed from each other, and from all other groups. Prophylactic SR-3 treatment was effective against the effects of both acute (779.50) and chronic (746.83) challenge with Ro4-1284 (Fig. 2).

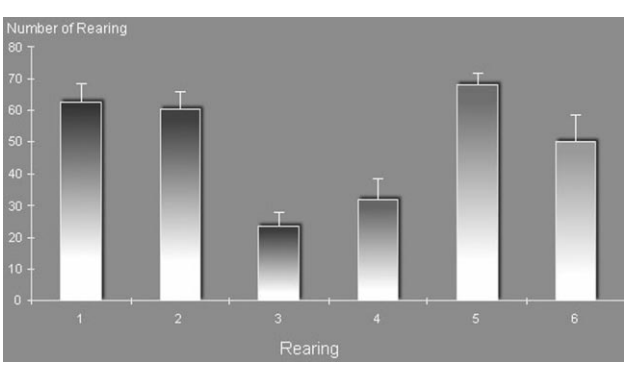

Fig. 3. Effects of Ro4-1284 and SR-3 on the number of Rearings. Data are expressed as means ± S.D. Groups are identified by the same numbers as in Fig. 1. See text for significant statistical differences.

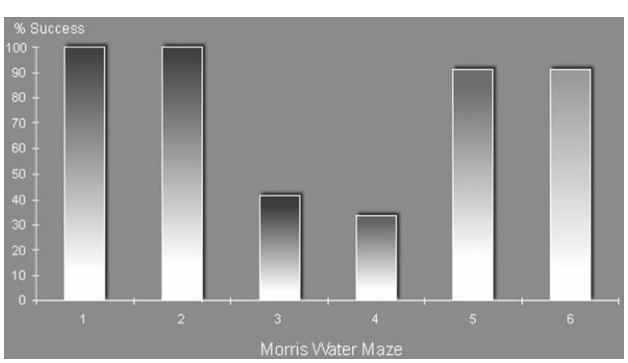

Fig. 5. Effects of Ro4-1284 and SR-3 on learning in the Morris Water Maze. Data are expressed as the percentage of successful rats in each group.

Table 1
Effects of Ro4-1284 and S-3 on the profile of free acids in synaptosomes prepared from the frontal cortex

	Acute			Chronic		
	Saline	Ro4-1284	+SR-3	Saline	Ro4-1284	+SR-3
14:0	1.5 ± 0.6	1.6 ± 0.6	1.2 ± 0.4	1.5 ± 0.7	2.3 ± 1.1	1.2 ± 0.6
16:0	21.5 ± 2.6	20.9 ± 2.8	21.7 ± 2.8	22.0 ± 2.4	24.2 ± 2.9	21.2 ± 2.5
18:0	26.4 ± 0.9	26.8 ± 0.8	21.9 ± 1.8	26.0 ± 1.0	27.0 ± 1.3	22.0 ± 1.9
18:1 ($n-9$)	24.5 ± 1.9	24.9 ± 2.1	26.0 ± 1.0	24.1 ± 1.8	25.9 ± 1.8	25.9 ± 1.2
18:2 ($n-6$)	0.9 ± 0.4	0.6 ± 0.5	1.8 ± 0.7	0.8 ± 0.5	0.4 ± 0.2	1.7 ± 0.8
18:3 ($n-3$)	1.2 ± 0.5	1.1 ± 0.5	3.6 ± 1.6	1.1 ± 0.6	0.5 ± 0.1	4.0 ± 1.5
20:0	0.2 ± 0.1	0.2 ± 0.1	0.02 ± 0.01	0.2 ± 0.1	0.3 ± 0.1	0.02 ± 0.01
20:3 ($n-6$)	2.0 ± 0.8	2.9 ± 0.9	2.5 ± 0.8	2.1 ± 0.9	2.0 ± 1.5	2.6 ± 0.9
20:4 ($n-6$)	4.2 ± 1.3	4.1 ± 1.3	3.0 ± 1.4	3.9 ± 1.5	2.9 ± 1.6	3.5 ± 2.0
21:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.3 ± 0.1
22:1	2.5 ± 0.7	2.6 ± 0.6	1.9 ± 1.6	2.0 ± 1.8	3.0 ± 1.2	2.0 ± 1.8
22:4 ($n-6$)	2.6 ± 0.4	2.7 ± 0.5	3.6 ± 1.6	2.3 ± 1.5	1.5 ± 0.7	3.0 ± 1.5
22:6 ($n-3$)	11.1 ± 1.0	11.3 ± 0.8	12.5 ± 0.9	11.0 ± 1.2	9.1 ± 1.0	13.1 ± 1.4
Total FA	2.5 ± 1.0	2.2 ± 0.9	3.6 ± 1.2	2.6 ± 1.1	1.6 ± 0.8	3.3 ± 1.2
S/US	1.10	1.10	0.74	1.20	1.00	0.87
Cholesterol	6.9 ± 2.2	6.8 ± 2.3	4.0 ± 1.8	6.5 ± 2.0	9.0 ± 2.0	6.3 ± 2.2

Values of all FA are expressed as percentage of total FA composition and given as means ± S.D. ($n = 12$). Total FA is expressed as a percentage of frontal cortex weight.
Cholesterol is expressed as promile of frontal cortex weight.
For significant statistical difference, see text.

3.4. Rearing

Rearing activity was almost entirely abolished in the chronic Ro4-1284 group (23.5) while the acute treatment significantly decreased rearing activity (32.0) compared to that of untreated rats (62.5). SR-3 treatment was effective against the effects of both acute (62.5) and chronic (50.33) challenge with Ro4-1284, although the rearing level for the chronic Ro4-1284 animals still remained significantly lower than the control level (Fig. 3).

3.5. Body temperature

Acute and chronic administration of Ro4-1284 induced intense hypothermia (about 29°C), and this effect was prevented by prior SR-3 treatment for both acute (36.39°C) and chronic (36.54°C) challenge with Ro4-1284 (Fig. 4).

3.6. Morris Water Maze

All 12 rats in both control groups (acute and chronic saline) reached the learning criterion while only five rats in the acute Ro4-1284 group and four rats in the chronic Ro4-1284 group succeeded. Pretreatment with SR-3 enabled 11 of the 12 Ro4-1284-treated animals to satisfy the criterion. The differences among groups were highly significant ($df = 5$, chi-square$[6 \times 2] = 28.65$, $P < 0.001$) (Fig. 5).

3.7. Fatty acid profile and cholesterol level

Chronic treatment (but not acute) with Ro4-1284 reduced the total level of FA in the membrane (2.6 to 1.6, $P < 0.001$) as well as the total level of polyunsaturated fatty acids. Acute administration of Ro4-1284 decreased the level of linoleic acid ($n-6$) ($P < 0.005$) without modifying the level of other polyunsaturated fatty acids. Significant changes ($P < 0.001$) were noted following chronic administration of Ro4-1284, namely reduced levels of $n-6$ FA [linoleic acid (18:2) arachidonic acid (20:4) and (22:4)], $n-3$ FA [α-linolenic (18:3), and docosahexaenoic acid (22:60)]. The level of cholesterol remained the same for the acute Ro4-1284 treatment group even after pretreatment with SR-3. A prominent increase in cholesterol level was found in chronic Ro4-1284-treated rats, and pretreatment with SR-3 normalized both the FA profile and the cholesterol level (Table 1).

4. Discussion

Ro4-1284 had profound deleterious effects on DA-mediated behaviors, including performance in two types of learning task (passive avoidance and Morris Water Maze), on motor activity (line crossing and rearing), and on thermoregulation. These behavioral and physiological changes were accompanied by an altered FA profile and an increase in the cholesterol level in the neuronal membrane. The results of this study also confirmed earlier observations that pretreatment with SR-3 can effectively prevent most of the acute and chronic damaging effects of Ro4-1284 on both learning and brain biochemistry.

The effects of SR-3 and Ro4-1284 on the DA level in the striatum were investigated in four independent groups of animals (six rats in each group). The groups were: (1) 3 weeks saline, (2) 3 weeks saline and 10 days Ro4-1284

from the 22nd day, (3) 3 weeks SR-3 and 10 days Ro4-1284 from the 22nd day, and (4) 3 weeks SR-3. The level of dopamine (nmol/g wet tissue) in the striatal synapotosomes was as follows: Group 1—54.8 ± 5.6; Group 2—21.6 ± 3.5; Group 3—50.3 ± 5.2; Group 4—53.8 ± 6.7 [two-way ANOVA, $F(3.20) = 45.19$, $P = 0.0001$]. This data indicates that SR-3 per se had no effect on the DA level. However, SR-3 prevented the decrease in DA level induced by Ro4-1284. These findings support the behavioral results. The metabolites of DA and DA receptor density and affinity were not measured and so no statement about the effects of SR-3 on DA metabolism can be made.

The hypothermia induced by Ro4-1284 in this study confirmed an earlier finding (Porsolt et al., 1979) and the ability of SR-3 to restore normal thermoregulation has also been reported previously (Yehuda and Carasso, 1993).

The brain dopaminergic system mediates many functions. The expectation is that DA depletion will affect all DA-mediated functions. In our study all dopaminergic mediated functions were affected by DA depletion. However, in some cases, not all DA-mediated functions changed in the same direction. The specificity of the effect was consistent with the findings of a recent study that demonstrated that nucleus accumbens 6-OHDA lesions (which induce DA depletion) influenced motor activity but not operant learning (Liu et al., 1998).

The effects of Ro4-1284 on water maze learning reflect disruptions of memory associated with hippocampal function. Although hippocampal acetylcholine is the major neurotransmitter in Morris Water Maze learning (Brandeis et al., 1989), hippocampal DA D_1 receptor antagonists (Hersi et al., 1995) and DA depletion (Gasbarri et al., 1996) produce learning and memory impairment.

Ro4-1284, in particular, has already been shown to impair PA learning (Kulkarni and Bocknik, 1973; Burkard et al., 1989). Dopamine D_1, D_2 and D_3 interact in PA learning (Cabib et al., 1996; Sigala et al., 1997). In our study, the learning deficits in acute and chronic Ro4-1284 treated rats cannot be explained entirely by the reduced level of motor activity. Passive avoidance was also reduced even though the unsuccessful animals did not differ from their successful counterparts.

Reduced dopaminergic activity resulted in hypomotility and a decrease in rearing frequency. It has been shown repeatedly that striatal DA (mainly acting at D_2 receptors) is the key neurotransmitter in locomotion. In contrast, control of other motor activity, such as rearing, is attributed to the nucleus accumbens D_1 dopaminergic system (Svenningsson et al., 1997; Swanson et al., 1997). Both hypomotility and reduced rearing seem to be affected by Ro4-1284, as has earlier been reported by Burkard et al. (1989).

To propose that SR-3 has a direct effect on the dopaminergic system by reason of its ability to prevent most of the damaging effects of Ro4-1284 is at best tentative. The failure of SR-3 to restore a normal level of rearing activity in the chronic Ro4-1284 group needs critical examination. The explanation might be that SR-3 has different effects on different parts of the dopaminergic system. It may be that SR-3 is effective in restoring D_2 mediated functions and less effective in restoring D_1-mediated functions. It is interesting to note that the reduced level of rearing induced by EAE treatment was not entirely reversed by SR-3 although the hypomotility was (Yehuda et al., 1997c). It must also be recalled that SR-3 has been shown to restore a wide range of impairments in cognitive and motor functions brought about by iron deficiency, cholinergic or serotonergic neurotoxins, or by an elevated cortisol level. The common denominator seems to be the neuronal membrane, and receptors are certainly part of the membrane.

The mode of action of SR-3 is still unknown. However, SR-3 has been shown to increase the level of free fatty acids in general and of polyunsaturated fatty acids in particular and to decrease the level of cholesterol in the neuronal membrane. In addition, the effects of SR-3 lead to structural and functional changes. The improvement in learning capacity might be attributed to the normalization of brain lipids.

In conclusion, this study indicates that DA depletion has a profound effect on lipid biochemistry in the brain, on aspects of motor activity, and on performance in two models of learning. SR-3 has differential beneficial effects on various dopaminergic systems. Taken together with other known effects of SR-3, it seems that the ability of SR-3 to act on the neuronal membrane explains the mode of action of the drug.

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