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A new polyamine derivative, a structural analog of spermine, with in vivo activity as an inhibitor of ethanol appetite

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Abstract—This report describes the design and synthesis of the synthetic polyamine DCD (*N*,*N*′-bis-(3-aminopropyl)cyclohexane-1,4-diamine, tetramethanesulfonate), a structural analog of spermine, and its in vivo activity as an inhibitor of alcohol consumption in a free-choice paradigm carried out on genetically high-ethanol-consuming UChB rats. After acute treatment with DCD (daily single dose, 20 mg/kg, p.o., 3 days), a 19% decrease in ethanol intake was obtained, without affecting the levels of food and water intake. After chronic treatment (daily single dose, 20 mg/kg, p.o., 60 days) a decrease of up to 60% in ethanol intake with respect to the basal period was provoked; this effect was significantly maintained during the post-treatment period and, according to the data obtained from the determination of acetaldehyde levels in blood, was not related to a possible disulfiram-like effect. The design of this new compound was carried out using molecular modeling techniques, with the structures of natural polyamines (putrescine, spermidine, and spermine) and biosynthetically related diamines (1,3-diaminopropane; DAP) as templates. These polyamines have shown activity as inhibitors of ethanol appetite in the same experimental model.

1. Introduction

Alcohol dependence affects the biological, psychological, and social aspects of personal life. The therapies developed for treating this problem generally consist of using drugs affecting different mechanisms of action, which attenuate the psychoactive effects of alcohol and reduce cravings for this substance. These drug associations attempt to reduce the manifestations of abstinence (using benzodiazepines and other tranquilizers), prolong and maintain the period of abstinence (adversive compounds, such as disulfiram[1] and acamprosate[2] or cyanamide,[3,4] or other compounds, such as naltrexone[5]), and contribute to the treatment of problems such as anxiety or depression,[6,7] which frequently accompany the problem of alcohol dependence.[8–10]

From the therapeutic point of view, recent advances made in the neurobiological area permit identification of the neurotransmitters involved in the establishment and maintenance of alcoholism[11,12] and show that alcohol impacts multiple neurochemical receptors in the central nervous system. Several studies showed that ethanol is a potent and selective inhibitor of the *N*-methyl-D-aspartate receptors (NMDAr) and that prolonged exposure to ethanol leads to a compensatory 'up-regulation' of these receptors, resulting in enhanced NMDA receptor-mediated functions after the removal of ethanol. It is assumed that these alterations contribute to the development of a tolerance and dependence of ethanol as well as the acute and delayed signs of ethanol withdrawal.[13,14]

NMDAr are large hetero-oligomeric complexes including at least two copies of an NR1 subunit and two copies of an NR2 subunit,[15] with a group of modulating sites, among which the polyamine site is exposed.[16–18]

The endogenous polyamines, spermidine (SPD) and spermine (SP) and their diamine precursor, putrescine

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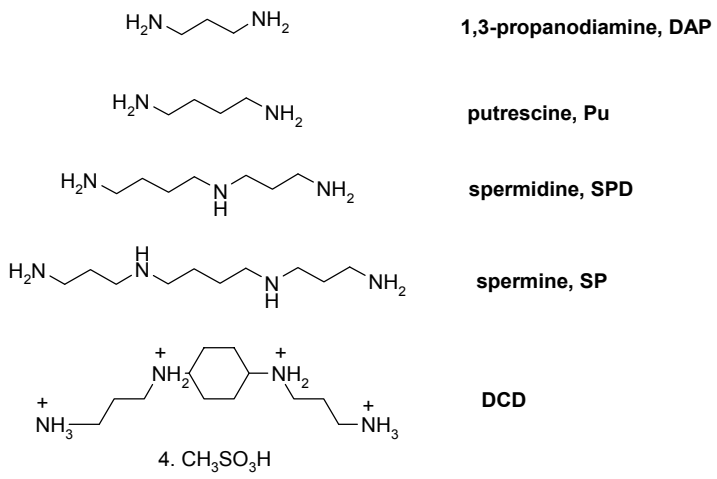


Figure 1. Structures for reference and DCD compounds.

(Pu), (Fig. 1) are intracellular cationic molecules that are essential for the growth, division, and differentiation of cells.[19,20] SPD and SP modulate the NMDA-receptor complex, with low micromolar levels potentiating and high micromolar concentrations inhibiting its function.[21,22] The affinity of the polyamines for many types of receptors and ion channels at which ethanol is also believed to act suggests many potential interactions among these agents.[23,24] In addition, interactions between ethanol and polyamines are of potential importance with regard to many of the sequels of ethanol abuse, including different states of ethanol withdrawal. NMDA-receptor-mediated mechanisms may be crucial in alcoholism and they provide a target for novel anti-alcoholism drugs.[25,26]

These facts raise the possibility of developing new effective compounds for the treatment of alcoholism, which would act by interacting with this NMDA receptor through polyamine-sensitive sites.

In a previous phase of our work, we demonstrated the in vivo activity of a series of natural polyamines, such as SPD and SP, and diamines, such as PU, and related compounds, such as 1,3-propanodiamine (DAP, Fig. 1), as inhibitors of ethanol appetite in a genetically alcoholic rat model. The activity shown by these compounds was significant, but with a limited time of action. This conclusion was based on the fact that a fast recovery was observed once the treatment period ended.[27]

Keeping in mind the aforementioned, new compounds were designed which would hopefully maintain the target biological activity, the inhibition of alcohol appetite, as well as possess an improved biological profile and a more favorable level of toxicity.

2. Design considerations

The pharmacology of polyamines has been reviewed quite extensively and this has contributed to the establishment of structure–activity relationships. Considering the different effects that these compounds produce on the NMDAr, initially, it appears that the activity is controlled by a wide range of factors.[28] Therefore, since the endogenous amines contain at least two amino groups, these factors can include (a) the total number of amino groups, (b) the number of carbon atoms that separate these groups, (c) the distance between the amino groups, and (d) the steric surroundings of these groups. The basicity of the amino groups as well as other properties, such as the total steric volume, lipophilia, and polarity, are factors that should be taken into consideration.

With regard to the structural factors, it has been demonstrated that diamine compounds with chain lengths between C2 and C3 are partial agonists, while those that have a chain length between C4 and C7 act as selective antagonists. Compounds with longer chains act as reverse agonists. For total agonism, the required interaction takes place with three amine-type points, two of which are situated within a distance of approximately 5 Å from each other, with the third point being at a distance of 5–6 Å from the other two points. While a coordinated action involving all three sites might explain the partial agonism found for diamines of shorter length, it was suggested that the antagonism may be attributed to an interaction with only two of the three sites. A fourth amine point of interaction, situated at an approximate distance of 12 Å, would be necessary in order to explain the reverse agonism of the long-chained diamines.[29]

Considering those previous data and in order to design new compounds, an indirect drug design approach was used. Following this approach, and starting from the analysis and characterization of the de novo tri-dimensional models (*InsigthII* software on *SiliconGraphics* workstations) of the diamines DAP and Pu as well as the polyamines SPD and SP, selected as the templates, a series of elements that make up the pharmacophore[30] were determined. Keeping in mind the polycationic character of the molecules under study and the data obtained from the reference literature, the descriptors considered for the design were (a) distance between the nitrogens present, (b) value of the charge and the distance between charged nitrogens, (c) values, distances, and geometric relationships among the molecular elec-

Table 1. Decrease in ethanol intake (%)[a] for the reference compounds and DCD

Compound	T-Basal	
	0–6 h	0–24 h
DAP	−32.7**	−21.0**
PU	−27.6**	−23.7**
SPD	−29.1**	−16.5*
SP	−42.3**	−27.9**
DCD	−19.3**	−6.09*

Acute treatment (20 mg/kg, p.o. × 3 days).
[a]Values are means (n = 8) of % changes of ethanol intake during the first 6 h (0–6 h) and the whole day (0–24 h) following drug administration, ** p <0.005, * p <0.05, with relation to ethanol intake during the basal period (paired Student's t-test).

Table 2. Descriptors obtained for the reference diamines DAP, PU, and DAC (AM1 over the more stable conformations[a])

REF	Distances N_1–N_2 (Å)	Charge		MEP[b]	
		N_1	N_2	N_1	N_2
DAP	3.37–5.03	−0.35	−0.34	−109.65	−109.85
PU	4.93–6.27	−0.35	−0.34	−113.56	−110.80
DAC	5.70	−0.33	−0.33	−113.27	−113.27

DAP: 1,3-propanodiamine; PU: putrescine; DAC: cyclohexane-1,4-diamine.
[a] See Experimental for details.
[b] Molecular electrostatic potential.

trostatic potential MEP, and (d) localization of HOMO and LUMO orbitals.

With regard to the in vivo activity shown by DAP and Pu (Table 1), these structures were taken to be active minimum fragments. For the design of new compounds, an additional approach has been made in which SP can be considered a central Pu with two lateral propylamine fragments. Following this approach, a preliminary structural modification was carried out, replacing the central Pu by another structural element, such as 1,4-diaminocyclohexyl, DAC, which maintains the previously cited descriptors within the permitted limits (Table 2). A new SP analog, *N*,*N*′-bis-(3-aminopropyl)cyclohexane-1,4-diamine (DCD, Figs. 1 and 2) was proposed as a target of synthesis.

3. Chemistry

The synthesis of DCD was carried out following Scheme 1. Basically, the synthesis occurs upon addition of the two nucleophilic centers of the starting product, 1,4-cyclohexanodiamine, to the proximal carbon of acrylonitrile, and after subsequent reduction of the intermediate by means of catalytic hydrogenation with Ni-Raney as the catalyst. The product, DCD, obtained as the free base, was subsequently treated in order to obtain the corresponding methanosulfonic salt derivative. The structural elucidation and characterization of the products have been carried out by infrared and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. The purity of the products was determined by C.H.N. elemental

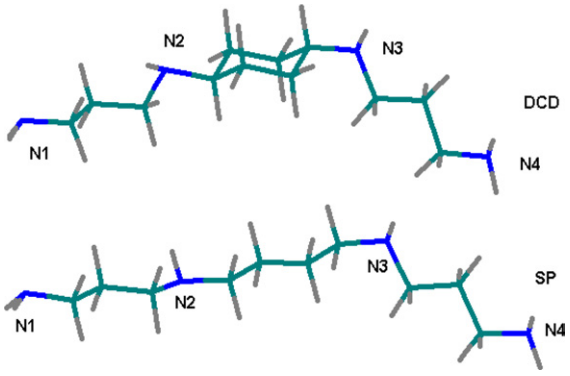
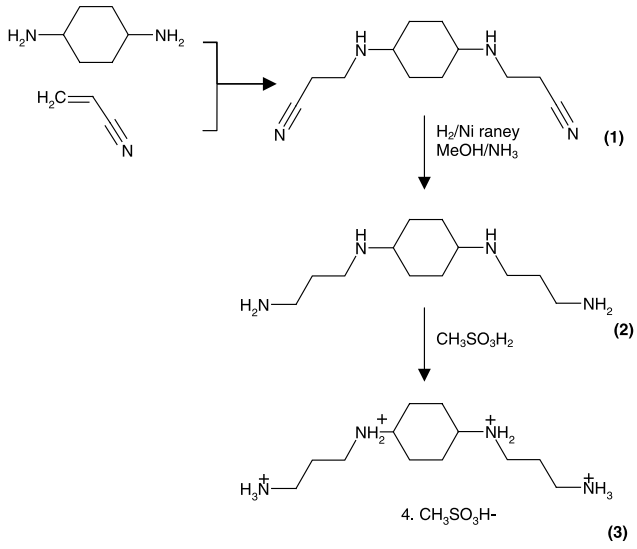


Figure 2. 3D-models for DCD and SP.



Scheme 1.

analysis and HPLC chromatography. The melting points of the solids were determined as complementary data.

4. Biological evaluation

4.1. Inhibition of ethanol appetite

In order to evaluate the target activity, we studied the effects of DCD and the reference endogenous polyamines on alcohol consumption in a free-choice paradigm carried out on genetically high-ethanol-consuming UChB rats; this strain has innate tolerance to ethanol,[31,32] due to genetic differences, including changes in acetaldehyde metabolism.[33–35] In the first series of experiments, the effect of acute treatment on ethanol, water, and food consumption was established using the selected compounds (DAP, Pu, SP, SPD, DCD). For this assay, three periods of tests were considered: (a) the 3-day basal period, during which basal consumption was established; (b) the 3-day treatment period, during which a single daily dose of 20 mg/kg of the compound was administered p.o. (vehicle in the control group) and ethanol intake was measured daily during the first 6 h (0–6 h) as

well as throughout the whole day (0–24 h), establishing time zero as the hour at which the compound was administered; and (c) the post-treatment period, which covers the days immediately following the treatment period.

In a second experiment carried out on different groups of UChB rats, the variation in ethanol, water, and food intake throughout a long-term administration of DCD was studied. For this assay, once a 3-day basal period of intake was established, the rats were subjected to a 60-day treatment period, during which a single daily dose of 20 mg/kg of DCD was administered p.o. Intake of ethanol, water, and food was measured once daily throughout the 0–24 h period in relation to time zero. The post-treatment period for this assay was extended long enough to assure recovery from DCD treatment.

4.2. Disulfiram-like effect

The search for an ideal drug for the treatment of alcoholism is principally centered on compounds that lack adverse effects, meaning disulfiram-like effects or effects linked to the blocking of the acetaldehyde dehydrogenase enzyme (ALDC), the enzyme following alcohol dehydrogenase in the major pathway of alcohol metabolism. Disulfiram inhibits ALDC, blocking oxidation of alcohol and allowing acetaldehyde to accumulate in the blood; accumulation of acetaldehyde produces the highly unpleasant disulfiram–alcohol reaction (flushing, throbbing in head and neck, throbbing headaches, respiratory difficulty, nausea, copious vomiting, sweating, thirst, chest pain, palpitations, dyspnea, hyperventilation, tachycardia, hypotension, syncope, weakness, vertigo, blurred vision, confusion) that deters consumption of alcohol.[36,37]

In order to discard the possibility that the inhibitory effect on ethanol appetite shown by compound DCD and reference polyamines might be related to possible inhibitory activity of ALDC, a study was carried out on additional groups of UChB rats, which were administered (i.p.) a standard dose of ethanol (2.76 g/kg) preceded by p.o. administration of 20 mg/kg of polyamine or a standard 300 mg/kg dose of disulfiram 30 min before the ethanol challenge. Blood was drawn from the tail vein and the acetaldehyde levels were determined by gas chromatography.

5. Results and discussion

5.1. Inhibition of ethanol appetite (acute and chronic treatment)

The results obtained in these assays are shown in Tables 1, 3, and 4. With respect to the activity shown with *acute treatment* (Table 1), all of the compounds tested significantly decreased ethanol intake in the UChB rats; the effects were more marked during the first 6 h after drug administration (0–6 h interval) as compared to those observed during the whole day (0–24 h interval). No significant variations were observed with regard to food and water intake.

In this assay, upon comparing the activity shown by DCD with that shown by SP, it was observed that recuperation from ethanol intake was faster with DCD.

Table 3. Effect[a] of DCD (chronic p.o. administration, 20 mg/kg × 60 days) on ethanol intake (measurement interval: 0–24 h)

Basal		Cycle									
		1	2	3	4	5	6	7	8	9	10
Mean	9.2	7.9	8.1	8.0	6.9	7.3	6.5	6.2	6.5	5.6	5.3
SE	0.62	0.89	0.86	0.63	0.83	0.77	0.78	0.61	0.40	0.65	0.81
%		**−14.13**	**−11.96**	**−13.04**	**−25.00**	**−20.65**	**−29.35**	**−32.61**	**−29.35**	**−39.13**	**−42.39**
		11	12	13	14	15	16	17	18	19	20
Mean	9.2	3.6	4.6	4.3	4.0	3.3	3.2	3.6	4.1	3.0	3.7
SE	0.62	0.70	0.77	0.58	0.73	0.52	0.59	0.41	0.33	0.49	0.16
%		**−60.87**	**−50.00**	**−53.26**	**−56.52**	**−64.13**	**−65.22**	**−60.87**	**−55.44**	**−67.39**	**−59.78**

[a] Values are means ± SEM ($n = 8$) of changes of daily ethanol intake, expressed as ml/100 g/day, in relation to the basal values.

Table 4. Effect[a] of DCD (chronic p.o. administration 20 mg/kg × 60 days) on water intake (measurement interval: 0–24 h)

Basal		Cycle									
		1	2	3	4	5	6	7	8	9	10
Mean	2.9	2.9	3.0	2.5	2.5	2.8	3.1	3.3	3.4	4.1	4.1
SE	0.76	0.64	0.72	0.64	0.76	0.72	0.67	0.53	0.47	0.41	0.80
%		**0**	**3.45**	**−13.79**	**−13.79**	**−3.44**	**6.89**	**13.79**	**17.24**	**41.38**	**41.38**
		11	12	13	14	15	16	17	18	19	20
Mean	2.9	4.0	4.5	4.9	4.5	4.7	5.5	4.6	4.6	4.9	5.8
SE	0.76	0.67	0.70	0.67	0.36	0.37	0.60	0.54	0.55	0.63	0.59
%		**37.93**	**55.17**	**68.96**	**55.17**	**62.07**	**89.66**	**58.62**	**58.62**	**68.92**	**100**

[a] Values are means ± SEM ($n = 8$) of changes of daily water intake, expressed as ml/100 g/day, in relation to the basal values.

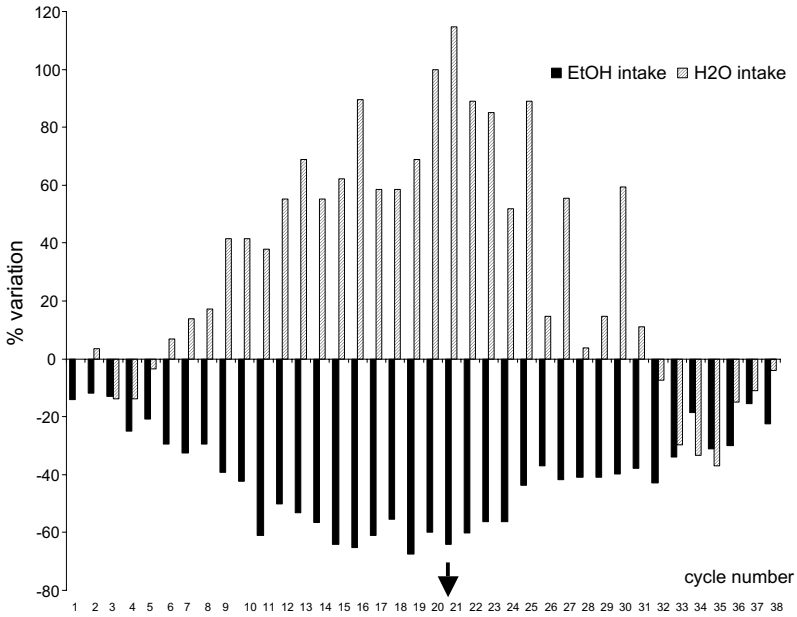


Figure 3. Percentage change of ethanol and H_2O intake during and after chronic DCD treatment (20 mg/kg, p.o.). DCD was administered daily from day 0 (cycle 1) to day 60 (cycle 20), as indicated by the arrow. Values are means of daily intake with relation to basal values.

Therefore, a new study was performed. The study involved single daily administration (p.o.) of 20 mg/kg of DCD (*chronic treatment*) for 60 days. Intake volume was measured in 3-day cycles (1–20, for the T period and 21–38 for the PT period). The data obtained appear in Tables 3 and 4 and in Figure 3. The decrease in ethanol intake (Table 3) was highly significant, reaching a maximum value of approximately 67%, and a gradual recovery of basal scores of ethanol intake occurred after stopping the treatment. However, in spite of the prolonged PT period, the basal values of intake were not recuperated.

Table 4 shows that long-term treatment with DCD greatly influenced water intake in the rats. According to the free-choice paradigm used, more than 70% of the total liquid intake was alcohol solution, and taking into account that the decrease in the volume of ethanol ingested was accompanied by an increase of water intake, it could be considered that there was no notable modification in the total volume of liquids ingested. No significant variations with regard to food intake were observed.

Daily observation of the animals during the chronic treatment with DCD revealed no detectable changes in exploratory behavior and mobility of the animals.

5.2. Disulfiram-like effect

Table 5 shows the results corresponding to the acetaldehyde levels found in the blood samples obtained from animals treated with different reference polyamines (PU, SPD, and SP, 20 mg/kg p.o.) and DCD (40 mg/kg p.o.). As a reference for this test, a standard dose (300 mg/kg) of disulfiram is administered, to which the alcohol consumption being practically reduced to zero in UChB rats.[35]

Table 5. Acetaldehyde levels (μg/100 ml) in blood[a]

Compound	Dose (mg/kg, p.o.)	Time (h)[b]		
		0.5	1	2
Vehicle[b]	—	53 ± 5	49 ± 6	18 ± 5
PU	20	83 ± 7	45 ± 6	35 ± 3
SPD	20	138 ± 21	155 ± 24*	42 ± 22
SP	20	204 ± 4*	50 ± 14	44 ± 8
DCD	40	32. ± 3	30 ± 2	29 ± 4
Disulfiram	300	725 ± 82**	816 ± 53**	594 ± 65**

[a] See Experimental for details.
[b] After ethanol administration.
* $p < 0.05$.
** $p < 0.005$.

As expected, after ethanol administration, a significant increase in the acetaldehyde levels was observed in the group treated with disulfiram, while there were only slight enhancements of acetaldehyde concentration in the groups treated with the different polyamines; said enhancements were very similar to those observed in the control group and were considered nonsignificant. In the case of DCD, the acetaldehyde levels detected were lower than those corresponding to the control group.

These results indicate that the inhibitory activities of voluntary ethanol consumption in UChB rats of the polyamine compounds studied are not related to ALDC enzyme inhibition.

6. Conclusions

Based on the data regarding inhibitory activity of ethanol appetite obtained for a group of endogenous polyamines, a structural analog, DCD, was designed. In this design, the butyl central fragment of SP was substituted by a cyclohexane ring.

The results showed that upon an acute 3-day treatment, DCD was able to reduce the ethanol intake in UChB rats (the decrease was higher during the 0–6 h interval) subjected to a free-choice paradigm, without significantly affecting water and food intake. The long-term treatment (60 days) with DCD provoked a decrease in ethanol consumption of approximately 67%. Concomitantly, the long-term DCD treatment also provoked a moderate but significant and reversible decrease of water intake.

Based on the data obtained it can be concluded that the inhibitory effect of DCD and the reference polyamines on ethanol intake is unrelated to an ALDC enzyme inhibition mechanism.

The reduction of ethanol intake by polyamines in UChB rats is possibly the result of NMDAr modulation in regions of the brain that are involved in the craving for different abuse substances, including alcohol.[38] It has been demonstrated that the endogenous polyamines can behave as NMDAr antagonists when administered at concentrations higher than the physiological levels.[39] The structural modification carried out on SP in order to obtain DCD presumably causes a modification of the logP of DCD with respect to the logP obtained for SP, which could favor the access of DCD to the target NMDA receptors.

7. Experimental

7.1. Molecular modeling

The de novo construction of the reference molecule models was carried out by applying the software package, InsightII,[40] on *SiliconGraphics* workstations. The protocol can be summarized as follows:

(a) Initial construction of the model.
(b) Hierarchized systematic conformational analysis: Determination of the rotation-sensitive bonds; election of a 30° window to check each dihedron. First filtration: elimination of the conformations that are indistinguishable by symmetry. Second filtration: elimination of conformations that present steric impediments. Third filtration: calculation of the energy of conformation and elimination of those conformations whose relative energy is greater than 10 kcal/mol at global minimum. Fourth filtration: optimization of the geometry of the conformation and elimination of those whose relative energy is greater than 10 kcal/mol at global minimum. All of the calculations were made within the framework of molecular mechanics, with the CVFF force field, (*Search and Compare* module, InsightII).
(c) Mechanoquantics optimization of the conformations obtained in the previous step, with the molecular orbital calculations package, *Mopac* (AM1[41] semi-empirical approach, *AMPAC/MOPAC* InsightII module).
(d) Obtainment of geometric descriptors, charges, and orbitals.
(e) Obtainment of MEP values and their geometric relationship (*MEPMIN* module in software package, MEPSIM 97[42]).

7.2. Synthesis

Chemicals were purchased from Aldrich, BDH Chemicals, Fluka, etc. All of the products of the intermediate and final reactions were characterized by infrared (Perkin Elmer FT 681) and nuclear magnetic resonance (Bruker AC-200e, 200 MHz, for ^{1}H and 50 MHz for C^{13}) spectroscopic techniques. The melting points were determined using a Mettler FP82 system equipped with a FP800/FP80 processor, an Olympus 8091 microscope, and a video system; they have not been corrected. Elemental analyses were obtained using a CHN-900 elemental analyzer (LECO). The interval of purity accepted was ±0.4%.

7.2.1. *N,N′*-Bis(2-cianoethyl)cyclohexano-1,4-diamine (1). Cyclohexane-1,4-diamine (5.7 g, 50 mmol, mixture of isomers, Aldrich 33,9980-9) was placed in a flask provided with both stirring and refrigeration systems and heated to 80 °C. Simultaneously, 7.23 ml (110 mmol) of acrylonitrile (BDH Chemicals 270471) was added dropwise, with energetic stirring. The mixture was maintained at 80 °C for 1 h, and then it was heated to 100 °C for 2 h. The mixture was allowed to cool and the solid was collected with ethyl ether, filtered, and purified. The desired compound (**1**) was obtained as a crystalline white solid (21.05 g; 95% yield). Mp = 102–105 °C (EtOH). IR (KBr) cm^{-1} = 3299 (N–H), 2925, 2852, 2800 (C–H aliphat.), 2245 (CN). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.94–1.49 (m, 6H, CH_2 and NH); 1.85–1.89 (m, 4H, CH_2); 2.40–2.46 (m, 6H, CH_2–NH_2 and CH); 2.84–290 (m, 4H, CH_2–NH_2). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 19.2 (CH_2CN); 31.9 (CH_2); 42.4 (CH–NH_2); 56.0 (CH); 118.7 (CN). C.H.N. ($C_{12}H_{20}N_4$) Calcd 65.49, 9.09, 25.45; Found: 65.00, 9.07, 25.52.

7.2.2. *N,N′*-Bis(3-aminopropyl)cyclohexano-1,4-diamine (2). A dissolution of *N,N′*-bis(2-cianoethyl)cyclohexano-1,4-diamine (**1**) in 150 ml of methanol saturated with NH_3 was placed in a Parr hydrogenating reactor provided with both stirring and refrigeration systems. Niquel-Raney (Fluka, 83440) was added (50 mg) and the mixture was maintained at 50 °C under hydrogen pressure (40–50 psi) for 6 days, with stirring periods of 8 h/day. The catalyst was eliminated by filtration and the solvent was eliminated under reduced pressure. The desired compound (**2**) was obtained as a greenish-colored syrup (5.48 g, 80% yield). IR (KBr) cm^{-1} = 3290, 3354 (NH_2 and NH), 2855, 2928 (C–H aliphat.). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 1.11–1.20 (m, 4H, CH_2); 1.48–1.66 (m, 10H, CH_2, NH_2 and NH); 1.92–2.00 (m, 4H, CH_2); 2.41–2.59 (m, 4H, CH_2); 2.65–2.79 (m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 31.9 (CH_2); 33.9 (CH_2); 40.27 (CH–NH_2); 44.9 (CH–NH); 58.7 (CH).

7.2.3. Tetramethanosulfonate of *N,N′*-bis(3-aminopropyl)cyclohexano-1,4-diamine (3). *N,N′*-Bis(3-aminopro-

pyl)cyclohexano-1,4-diamine (**2**, 2.14 g, 9.4 mmol) dissolved in 50 ml of methanol was mixed, under stirring, with 2.6 ml (40 mmol) of methanosulfonic acid (Aldrich, M-860-6) dissolved in 10 ml of ethyl ether. The mixture was stirred at room temperature for 2 h and then filtered, dried, and purified. Compound (**3**) was obtained as a white crystalline solid: (1.5 g, 25% yield). Mp = 215–217 °C (EtOH). IR (KBr) cm^{-1} = 2920, 2860 (C–H aliphat.); 1196 (SO_3); ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 1.80–1.90 (m, 4H, CH_2); 1.92–1.96 (m, 4H, CH_2); 2.14–2.20 (m, 4H, CH_2, NH_2); 2.24 (s, 12H, CH_3); 2.90–3.02 (m, 10H, CH_2 and CH); 7.84 (br s, 6H, NH^+_3 and NH^+_2), ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 23.6 (CH_2); 25.9 (CH_2); 36.0 (CH_2); 39.4 (CH_3); 40.0 (CH–NH_2); 54.3 (CH). C.H.N. ($C_{12}H_{28}N_4{\cdot}4CH_3SO_3H{\cdot}H_2O$) Calcd: 30.48, 7.30, 8.88; Found: 30.72, 6.99, 8.40.

7.3. Inhibition of ethanol intake

UChB Wistar male and female alcohol-naïve rats, approximately 90 days old at the beginning of the assay, were selected for their high level of voluntary consumption of aqueous ethanol solution (10% v/v). They were obtained from the animal breeding center at the University of Chile (Santiago de Chile, Chile). The rats were individually housed in standard steel cages with free access to an ethanol solution (10% v/v), water, and food. The temperature was maintained at 22 ± 1 °C, with periodic cycles of air changes. A regimen of light/darkness of 12 h each and a relative humidity of approximately 60% was also maintained.

All of the procedures regarding the care and use of animals and animal experimentation reported in this paper complied with norms set by our institutions and with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Throughout the duration of the experiment, alcohol was offered in the homecage. A two-bottle free-choice regimen between a 10% (v/v) alcohol solution and water was offered, with unlimited access for 24 h/day. The bottles were refilled every day with fresh solutions and their relative position was interchanged in order to avoid development of position preference. Standard chow pellets were available ad libitum throughout the study.

Rats (n = 8–10) were assigned to each experimental group in a manner that ensured that the groups did not differ in basal alcohol intake prior to drug treatment. Physiological serum was the administration vehicle. The doses chosen for natural polyamines are far below the acute oral toxic doses cited in the reference literature.[43]

The data regarding alcohol, water, and food intake were with reference to the time zero, the start of the dark phase of day 1 (at 19.00 h). Three periods of study were established: (a) the basal period, B, in which the animals were maintained under standard housing conditions, without receiving any treatment; daily measurements of ethanol, food, and water intake during this period were considered the basal values; (b) the treatment period, T, during which (3 days for the acute treatment and 60 for the chronic treatment) a single 20 mg/kg dose of the compound under evaluation was administered p.o. daily at time zero; the corresponding volume of the vehicle was administered p.o. to the control group; and (c) the post-treatment period, PT, consisted of the days immediately following the treatment period, in which the animals were maintained under basal conditions, without receiving any treatment.

In each 3-day period, ethanol intake was measured daily during the first 6 h as well for the whole day (0–24 h) in relation to time zero. The daily intake of water and food (0–24 h) was evaluated throughout the three periods. Daily intake measurements of ethanol, water, and food during the basal period normalized at ml/100 g/day for water and ethanol solution, and at g/100 g/day for food. Differences in ethanol, water, and food intake between the T and B periods, as well as between the PT and B periods, were calculated for each rat. The results were expressed as means ± SEM of changes in alcohol and water consumption, resulting from comparing the intakes during the T and PT period to those of the B period. The significance of the differences was established by means of the paired Student's t-test.

7.4. Disulfiram-like effect

UChB Wistar male and female alcohol-naïve adult rats (250–300 g) were obtained from the animal breeding center at the University of Chile (Santiago de Chile, Chile). They were distributed in three homogeneous groups (n = 6–8). Physiological serum (administration vehicle) was administered p.o. to the control group 30 min before ethanol administration (2.76 g/kg, i.p.). DCD (20 mg/kg) was administered p.o. to the second group 30 min before receiving the ethanol dose. Disulfiram (300 mg/kg) was administered p.o. to the third group 30 min before administration of the ethanol. Blood was drawn from the tail vein and the acetaldehyde levels were determined by gas chromatography ('Head-Space' method[44]). The significance of the differences was established by means of a one-way ANOVA followed by a Dunnett's test for multiple comparisons.

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