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Chlorpromazine-induced alterations in hypothalamic amine metabolism and stress responses in severe cold

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Summary. To investigate the effects on the central nervous system of severe cold stress with and without chlorpromazine, guinea pigs were treated with chlorpromazine or 0.9% NaCl and exposed to −20°C or +23°C for 1 h. Hypothalamic noradrenaline (NA), dopamine (DA), 5-hydroxy-tryptamine (5-HT), 3-methoxy-4-hydroxyphenyl ethylene glycol (MHPG), homovanillinic acid (HVA) and 5-hydroxy-indoleacetic acid (5-HIAA) were determined by high-performance liquid chromatography. Serum, urinary and vitreous fluid catecholamines, muscle and liver glycogen, and blood glucose were also measured. Chlorpromazine caused distinct hypothermia at −20°C and slight hypothermia at +23°C. The rise in hypothalamic MHPG, 5-HIAA and MHPG/NA and in 5-HIAA/5-HT ratios in the cold indicate increased noradrenergic and serotonergic activity. The latter was inhibited by chlorpromazine and a drug-induced inhibition of noradrenergic neurons could not be ruled out. Chlorpromazine increased the turnover of DA at room temperature and the same tendency was seen in the cold. The hypothermic animals had low serum catecholamines, indicating diminished sympathetic activity. The chlorpromazine-treated cold-exposed animals did not react to the environmental stress by sympathetic activation, as urinary NA and adrenaline were not elevated, but DA was excreted by all the drug-treated animals. Vitreous fluid NA and DA were elevated as an indicator of cold stress, and no drug effect was seen in this fluid.

Key words: Severe cold stress – Hypothermia – Chlorpromazine, hypothermia

Zusammenfassung. Um die zentralen Auswirkungen eines schweren Kältestresses unter und ohne Einfluß von Chlorpromazin zu untersuchen, wurden Meerschweinchen mit Chlorpromazin oder 0,9%igem NaCl behandelt und für eine Stunde entweder bei einer Umgebungstemperatur von −20°C oder

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+23°C gehalten. Die Konzentrationen an hypothalamischen NA, DA, 5-HT, MHPG, HVA and 5-HIAA wurden mit HPLC bestimmt. Die Katekolamine im Serum, dem Urin und dem Glaskörper, das Muskel- und Leberglykogen sowie Blutzucker wurden auch bestimmt. Chlorpromazin verursachte eine deutliche Hypothermie bei −20°C und eine geringe Hypothermie bei +23°C. Der Anstieg der hypothalamischen MHPG, 5-HIAA and MHPG/NA sowie 5-HIAA/5-HT in der Kälte indiziert eine noradrenergische und serotonergische Aktivität. Letztgenannte wurde durch Chlorpromazin gehemmt und eine Hemmung von noradrenergischen Neuronen, verursacht durch die Medikamente, konnte nicht ausgeschlossen werden. Chlorpromazin erhöhte den Umsatz von DA unter +23°C und dieselbe Tendenz war unter Kälteeinfluß festzustellen. Die hypothermischen Tiere hatten niedrige Katecholaminwerte im Serum, was eine verminderte sympathische Aktivität indiziert. Die mit Chlorpromazin behandelten Meerschweinchen mit Kälte-Exposition reagierten auf den Kältestreß nicht mit sympathischer Aktivierung; denn NA und Adrenalin wiesen im Urin keine erhöhten Werte auf. DA wurde von allen Chlorpromazin behandelten Tieren ausgeschieden. NA- und DA-Werte im Glaskörper waren als ein Indikator von Kältestreß erhöht und ein Arzneimitteleinfluß konnte in dieser Flüssigkeit nicht nachgewiesen werden.

Schlüsselwörter: Kältestreß – Hypothermie – Chlorpromazin, Hypothermie

Introduction

There are experimental and clinical data available on chlorpromazine and hypothermia [1, 2], but not much attention has been paid to this neuroleptic in connection with extreme cold. Chlorpromazine can be expected to cause a rapid drop in core temperature followed by death in severe cold, as the drug is a potent central nervous system depressor and hypothermic agent.

At ordinary ambient temperatures, chlorpromazine is a potent vasodilator because of its alpha-receptor-blocking effect on blood vessels, or it may decrease the sensitivity of the midbrain vasomotor center [3]. In severe cold, its vasodilatory action and the consequent increase in heat loss may not as a whole play a significant role, because severe cold itself is a potent stimulus of vasoconstriction, but vasodilation caused by the drug may lead to rapid heat loss during the initial phase of cold exposure [1].

It is known that chlorpromazine alters the concentrations of central monoamines and interactions with noradrenergic [4]; dopaminergic [5] and serotonergic [6] neurons have been reported in connection with thermoregulatory functions. On the other hand, stress-induced activation of central catecholamine metabolism has been documented in many studies [7].

The autopsy findings in hypothermia deaths are usually typical of severe stress, e.g., gastric mucosal bleeding and elevated urinary catecholamines [8], but in certain fatalities these signs are not present and thus the diagnosis of hypothermia can remain only speculative. As potent central nervous system de-

pressors, neuroleptic drugs may modify the stress responses of an organism even if the environmental conditions are severe, as in extreme cold stress, and in this way these drugs may complicate the determination of the cause and manner of death. According to this hypothesis we studied the effects of severe cold stress and chlorpromazine on hypothalamic monoamine metabolism in order to find out if the cold-induced activation of monoaminergic neurons was modified by the drug. At the same time, we tried to find out to what extent the sympathetic nervous system can react by studying the serum and urinary and vitreous fluid catecholamines under these conditions.

Materials and methods

Thirty-two male guinea pigs weighing 415–870 g were used in the experiments. The animals were housed at $+22 \pm 1°C$ and allowed free access to food pellets, vegetables and water. They were divided into four groups of eight each and treated in the following ways: group I received 20 mg/kg chlorpromazine (Largactil, Rhone-Poulenc) i.p. and was then immediately exposed to $-20°C$ in a cold chamber for 1 h; group III received the same dose of chlorpromazine and the animals were kept at $+23°C$ for 1 h.

Groups II and IV served as controls and received a corresponding volume 0.9% NaCl i.p. relative to body weight and were also exposed to $-20°$ or $+23°C$, respectively. The animals were put in a box built of hard plastic and equipped with metal pins that restricted their movements, thus allowing continuous temperature measurements, but no extra discomfort.

Colon temperature was measured continuously during the experiments by inserting a thermistor probe (Ellab) 6 cm into the colon, which was fixed by adhesive tape. Temperature readings were recorded every 5 min. The ear skin temperature was also measured continuously by fixing a thermistor probe to the ear lobe by adhesive tape, and the readings were recorded every 5 min. At $-20°C$, in both groups the ear lobes of the guinea pigs were frozen at the end of the experiment. Ear lobe temperature measurements were regarded as unnecessary in these groups, as pilot experiments showed that freezing occurred rapidly at $-20°C$.

Biogenic amines and their metabolites in the hypothalamus

The hypothalamus was quickly removed and weighed and homogenized in ice-cold 0.1 *M* $HClO_4$: 50 µl of preservation solution containing 0.02 *M* $Na_2S_2O_5$ and 0.01 *M* EDTA was added to the homogenate. The homogenate was centrifuged for 20 min by 20000 g at $+4°C$. Perchloric acid was added to each mg wet weight of hypothalamus, so that 20 µl homogenate represented 1 mg tissue content. The supernatant of the hypothalamus homogenate was diluted with H_2O 1:4 for biogenic amines and 1:2 for amine metabolites and these dilutes were used as final samples for measurements. The whole 50 µl sample loop was used as a sample size.

Serum, urinary and vitreous fluid catecholamines

The blood samples were placed in tubes containing 5 µl of 1 *M* $Na_2S_2O_5$ and cooled. They were then centrifuged for 8 min at 3000 *g* and the serum stored at $-70°C$ until analyzed. The urine samples were taken by needle from the bladder of each animal that had enough urine; 300 µl urine was acidified with 20 µl 1 *M* HCl and the samples stored at $-70°C$ until analyzed.

Serum and urine catecholamines were purified by an Al_2O_3 extraction procedure. The catechols were extracted at pH 8.60 from 30 µl of serum or urine into 30 mg Al_2O_3 in 5 ml conical test tubes with dihydroxybenzylamine (DHBA) and isoprenaline (ISO) as internal standards. After washing four times with 2 ml H_2O, the catechols were released into 100 µl of 0.2 *M* $HClO_4$; 50 µl or less was injected into the chromatograph. The ratio of peak height of

each catecholamine to the average peak heights of the internal standards were used as the basis for the concentration calculations.

Vitreous fluid was aspirated from both eyes with a syringe, centrifuged at 5000 *g* and the supernatant stored at −70°C until analyzed; 200 µl of the supernatant was taken for Al_2O_3 extraction as described above.

LC-EC method for biogenic amines and their metabolites

The modified liquid chromatographic electrochemical detection (LC-EC) method of Taylor et al. [9] was used for the determination of noradrenaline (NA), adrenaline (A), dopamine (DA) and 5-hydroxytryptamine (5-HT). For the metabolites 3-methoxy-4-hydroxyphenyl ethylene glycol (MHPG), homovanillinic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) the method of Scheinin et al. [10] was used.

The chromatograph consisted of a M-45 pump (Water Associates, Milford, MA, USA), a Rheodyne 7125 injector with a 50 µl sample loop, an Ultrasphere ODS 5 µl (150 × 4 mm) reversed phase analytical column (Beckman Instruments, Berkeley, Calif., USA) protected with a guard column (50 × 2 mm) filled with Vydac RP (30–40 µm), a LC-4B amperometric detector (Bioanalytical Systems Inc., West Lafayette, Ind., USA) with glassy carbon working electrode and an $Ag/AgCl_2$ reference electrode. An oxidation potential of 0.6 V was used. The chromatograms were recorded on a double channel recorder (Kipp & Zonen, Delft, Netherlands) and the peak heights used as the basis for the concentration calculations.

Liver and muscle glycogen; blood glucose

Liver and muscle glycogen were measured using the phenolsuphuric acid colorimetric method of Lo et al. [11]. An enzymatic test kit (Gluco Rapid Test, F. Hoffmann-La Roche, Basel, Switzerland) was used for blood glucose determinations.

Statistics

Statistical analysis was performed by computer using a one-way analysis of variance. Where the *F*-value indicated overall significance at the $P < 0.05$ level or greater, Bonferroni's modified *t*-test was used for the individual group comparisons.

Results

A drop in colon temperature was seen in both chlorpromazine-treated groups as compared with their control groups. This drop was 9.2° ± 0.6°C after 1 h of severe cold in the drug-treated animals, which was a significantly higher drop than in their controls, whose temperature dropped 5.6° ± 1.0°C ($P < 0.01$). At room temperature, the chlorpromazine-treated animals had a smaller drop (1.7° ± 0.3°C), but this, too, was significantly greater than in the corresponding controls, whose colon temperature remained around the same level throughout the experiment ($P < 0.01$). There were no statistically significant differences in the initial colon temperatures between groups (Figs. 1, 2).

Ear lobe temperatures in the guinea pigs kept at room temperature were higher in the drug-treated animals in the initial phase of the experiments, but not significantly so. After 10–15 min the chlorpromazine-treated animals still had a higher ear-lobe temperature, but the difference did not reach the level of significance. The value for these two groups then came closer to each other towards the end of the experiments (Fig. 3).

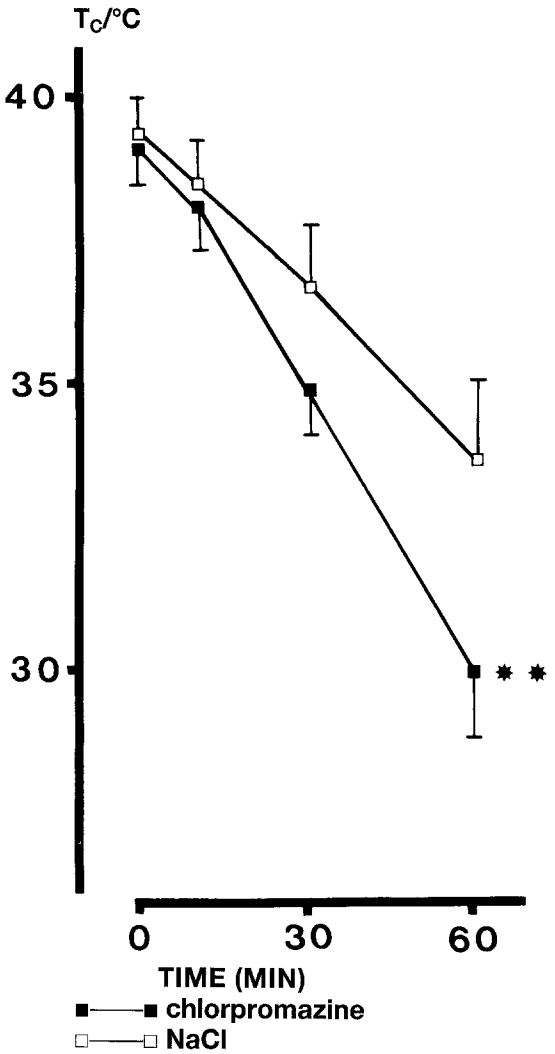

Fig. 1. Colon temperature of guinea pigs treated with chlorpromazine (20 mg · kg^{-1} i.p.) or 0.9% NaCl and exposed to −20°C for 1 h; $P < 0.01$, Bonferroni's test

Hypothalamic NA, DA, 5-HT and their metabolites MHPG, HVA and *5-HIAA*

Hypothalamic NA and MHPG. The two cold-exposed groups had significantly lower hypothalamic NA concentrations than the corresponding groups kept at room temperature ($P < 0.01$), while the concentrations were lower in the cold-exposed chlorpromazine-treated animals than in the cold-exposed controls. Here, though, the difference did not reach the level of significance. The drug-treated animals had a significantly lower hypothalamic NA concentration than their controls at room temperature ($P < 0.05$). The mean NA concentrations with statistical comparisons are presented in Table 1.

There were no statistically significant differences in hypothalamic MHPG concentrations between the chlorpromazine-treated groups and their controls.

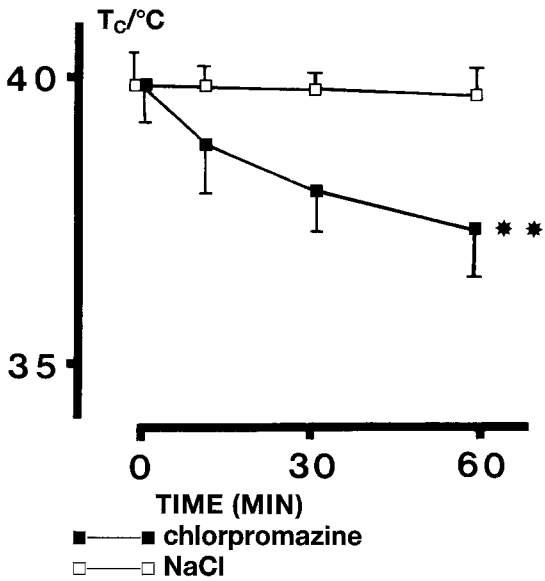

Fig. 2. Colon temperature of guinea pigs treated with chlorpromazine (20 mg · kg^{-1} i.p.) or 0.9% NaCl and kept at +23°C for 1 h; $P < 0.01$, Bonferroni's test

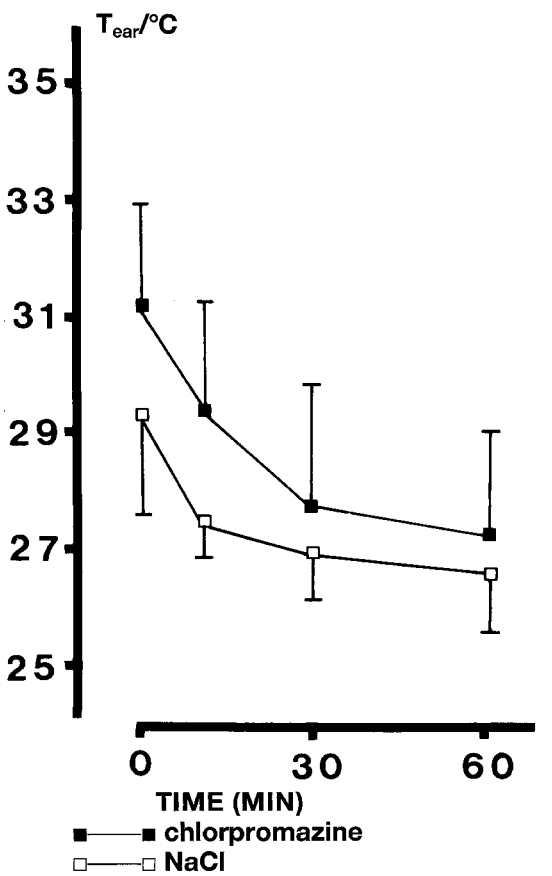

Fig. 3. Ear-lobe temperature of guinea pigs treated with chlorpromazine (20 mg · kg^{-1} i.p.) or 0.9% NaCl and kept at +23°C for 1 h

Table 1. Hypothalamic NA, MHPG and MHPG/NA ratio in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

Group		NA (ng · mg^{-1})	MHPG (ng · mg^{-1})	MHPG/NA
I	(CPZ, −20°C)	932.0 ± 139.5* ($n = 8$)	422.2 ± 131.7* ($n = 7$)	0.450 ± 0.132* ($n = 7$)
II	(NaCl, −20°C)	1109.3 ± 214.7** ($n = 8$)	416.9 ± 81.8** ($n = 8$)	0.400 ± 0.144** ($n = 8$)
III	(CPZ, +23°C)	1349.2 ± 243.0*** ($n = 7$)	3.8 ± 6.5 ($n = 6$)	0.003 ± 0.006 ($n = 6$)
IV	(NaCl, +23°C)	2022.2 ± 472.9 ($n = 8$)	6.7 ± 14.2 ($n = 8$)	0.012 ± 0.004 ($n = 8$)

Values are means ± SD. Statistical significance: * $P < 0.01$ compared to CPZ group kept at +23°C; ** $P < 0.01$ compared to NaCl group kept at +23°C; *** $P < 0.05$ compared to NaCl group kept at +23°C; Bonferroni's test

After 1 h at room temperature, the drug-treated guinea pigs and their controls had only very small amounts of MHPG in the hypothalamus. Both groups exposed to the cold had hypothalamic MHPG concentrations that were several hundred times higher than the controls (Table 1).

The hypothalamic MHPG/NA ratio was similar and high in both cold-exposed groups. The ratio was very low in the animals kept at room temperature, the difference being significant in comparison with the animals treated the same way and kept in severe cold ($P < 0.01$ for both pairs) (Table 1).

Hypothalamic DA and HVA. The hypothalamic DA concentrations in both groups exposed to severe cold were about the same, as were those in the two groups kept at room temperature. DA was higher in the chlorpromazine-treated cold-exposed animals than in the corresponding group kept at room temperature, although the difference was not significant. The NaCl-treated cold-exposed animals also had higher hypothalamic DA concentrations than the corresponding groups kept at room temperature, the difference being statistically significant ($P < 0.05$). These results are presented in Table 2.

Both chlorpromazine-treated groups had practically the same hypothalamic HVA concentrations, and there were also no statistically significant differences between the two NaCl-treated groups. HVA concentrations were higher in the cold-exposed chlorpromazine-treated animals than in their cold-exposed controls, but not significantly so. The drug-treated animals kept at room temperature had significantly higher HVA concentrations than their NaCl controls ($P < 0.01$). The hypothalamic HVA/DA ratio did not differ significantly between the groups (Table 2).

Hypothalamic 5-HT and 5-HIAA. There were no statistically significant differences in hypothalamic 5-HT concentrations between groups (Table 3), but the chlorpromazine group exposed to severe cold had a significantly lower amount of 5-HIAA in the hypothalamus than their controls ($P < 0.05$) whereas there

Table 2. Hypothalamic DA, HVA and HVA/DA ratio in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

Group		DA (ng · mg^{-1})	HVA (ng · mg^{-1})	HVA/DA
I	(CPZ, −20°C)	234.2 ± 60.9 ($n = 8$)	334.3 ± 79.5 ($n = 7$)	1.399 ± 0.502 ($n = 7$)
II	(NaCl, −20°C)	241.5 ± 54.2* ($n = 8$)	290.4 ± 89.7 ($n = 8$)	1.217 ± 0.285 ($n = 8$)
III	(CPZ, +23°C)	182.3 ± 32.7 ($n = 7$)	340.5 ± 37.6** ($n = 7$)	1.939 ± 0.157 ($n = 7$)
IV	(NaCl, +23°C)	166.6 ± 31.8 ($n = 8$)	211.8 ± 74.6 ($n = 8$)	1.355 ± 0.606 ($n = 8$)

Values are means ± SD. Statistical significance: * $P < 0.05$ compared to NaCl group kept at +23°C; ** $P < 0.01$ compared to NaCl group kept at +23°C; Bonferroni's test

Table 3. Hypothalamic 5-HT, 5-HIAA and 5-HIAA/5-HT ratio in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

Group		5-HT (ng · mg^{-1})	5-HIAA (ng · mg^{-1})	5-HIAA/5-HT
I	(CPZ, −20°C)	386.3 ± 72.8 ($n = 8$)	96.1 ± 33.4*, ** ($n = 7$)	0.251 ± 0.067 ($n = 7$)
II	(NaCl, −20°C)	455.8 ± 96.2 ($n = 8$)	149.6 ± 22.7*** ($n = 8$)	0.340 ± 0.082 ($n = 8$)
III	(CPZ, +23°C)	481.2 ± 145.5 ($n = 7$)	48.7 ± 18.2 ($n = 7$)	0.120 ± 0.040 ($n = 7$)
IV	(NaCl, +23°C)	514.1 ± 88.9 ($n = 8$)	53.4 ± 20.9 ($n = 8$)	0.103 ± 0.035 ($n = 8$)

Values are means ± SD. Statistical significance: * $P < 0.05$ compared to NaCl group kept at −20°C; ** $P < 0.01$ compared to CPZ group kept at +23°C; *** $P < 0.01$ compared to NaCl group kept at +23°C; Bonferroni's test

was no difference at room temperature. The cold-exposed chlorpromazine-treated animals had a significantly higher 5-HIAA concentration than those treated with the drug and kept at room temperature ($P < 0.01$), and the same situation prevailed in the cold-exposed and room temperature NaCl-treated groups ($P < 0.01$) (Table 2).

Serum, urinary and vitreous fluid catecholamines

Serum, NA and A. There were no statistically significant differences in serum NA concentrations between the chlorpromazine-treated groups and their controls. The cold-exposed chlorpromazine-treated animals had significantly lower serum NA concentrations than those treated with the drug and kept at room

Table 4. Serum catecholamines NA and A in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

	Group I (CPZ, −20°C)	Group II (NaCl, −20°C)	Group III (CPZ, +23°C)	Group IV (NaCl, +23°C)
Serum NA (ng · ml^{-1})	28.7 ± 8.0* ($n = 7$)	23.8 ± 13.7** ($n = 8$)	66.8 ± 27.6 ($n = 8$)	57.3 ± 28.3 ($n = 8$)
Serum A (ng · ml^{-1})	12.2 ± 9.0*** ($n = 7$)	32.8 ± 15.7 ($n = 8$)	71.4 ± 69.8 ($n = 8$)	105.7 ± 80.4 ($n = 5$)

Values are means ± SD. Statistical significance: * $P < 0.05$ compared to CPZ group kept at +23°C; ** $P < 0.05$ compared to NaCl group kept at +23°C; *** $P < 0.05$ compared to NaCl group kept at −20°C; Bonferroni's test

Table 5. Urinary catecholamines NA, A and DA in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

	Group I (CPZ, −20°C)	Group II (NaCl, −20°C)	Group III (CPZ, +23°C)	Group IV (NaCl, +23°C)
Urinary NA (ng · ml^{-1})	33.8 ± 25.9 ($n = 5$)	59.4 ± 38.9 ($n = 5$)	28.0 ± 37.2 ($n = 6$)	17.4 ± 6.5 ($n = 5$)
Urinary A (ng · ml^{-1})	12.5 ± 8.7* ($n = 5$)	171.4 ± 73.7** ($n = 5$)	33.3 ± 46.6 ($n = 6$)	11.2 ± 7.3 ($n = 6$)
Urinary DA (ng · ml^{-1})	8.2 ± 7.9 ($n = 5$)	1.6 ± 2.2 ($n = 5$)	5.5 ± 4.9 ($n = 5$)	0.8 ± 2.1 ($n = 6$)

Values are means ± SD. Statistical significance: * $P < 0.01$ compared to NaCl group kept at −20°C; ** $P < 0.01$ compared to NaCl group kept at +23°C; Bonferroni's test

temperature ($P < 0.05$), and the NaCl-treated guinea pigs exposed to severe cold also had significantly lower serum NA concentrations than their counterparts kept at room temperature ($P < 0.05$). These and other differences between the groups are presented in Table 4.

Serum A concentrations were significantly lower in the chlorpromazine-treated cold-exposed animals than in their controls ($P < 0.05$) and were also lower in the drug-treated group kept at room temperature than in the corresponding controls, but not significantly so. The cold-exposed drug-treated animals also had a lower mean serum A concentration than the drug-treated animals kept at room temperature, but this difference was not significant, and a similar nonsignificant relationship existed among the animals receiving NaCl (Table 4).

Urinary NA, A and DA. DA appeared in detectable amounts in every chlorpromazine-treated animal but in only some of the controls, the amount being slightly higher in the cold-exposed individuals. No significant differences were seen between the groups (Table 5). There were no significant differences in urinary NA concentrations between groups, but urinary A was significantly lower in the cold-exposed chlorpromazine-treated animals than in their controls

Table 6. Vitreous fluid catecholamines NA and DA in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

	Group I (CPZ, −20°C)	Group II (NaCl, −20°C)	Group III (CPZ, +23°C)	Group IV (NaCl, +23°C)
Vitreous fluid NA (ng · ml^{-1})	3.0 ± 1.1* ($n = 7$)	2.8 ± 1.6** ($n = 5$)	0.3 ± 0.3 ($n = 8$)	0.4 ± 0.1 ($n = 7$)
Vitreous fluid DA (ng · ml^{-1})	24.7 ± 15.1*** ($n = 7$)	11.9 ± 6.3**** ($n = 5$)	4.8 ± 1.3 ($n = 8$)	3.9 ± 0.6 ($n = 7$)

Values are means ± SD. Statistical significance: * $P < 0.01$ compared to CPZ group kept at +23°C; ** $P < 0.01$ compared to NaCl group kept at +23°C; *** $P < 0.05$ compared to CPZ group kept at +23°C; **** $P < 0.05$ compared to NaCl group kept at +23°C; Bonferroni's test

($P < 0.01$), although no significant differences were seen at room temperature. There was also no significant difference between the two chlorpromazine-treated groups. The cold-exposed NaCl-treated guinea pigs had significantly higher urinary A concentrations than the corresponding group kept at room temperature ($P < 0.01$) (Table 5).

Vitreous fluid catecholamines. Both cold-exposed groups had some NA in the vitreous fluid, more than the corresponding groups kept at room temperature, the difference being significant between both the two chlorpromazine groups ($P < 0.01$) and the two NaCl groups ($P < 0.01$). There were no significant differences between the drug-treated groups and their controls. Vitreous fluid DA concentrations were also significantly higher in both the drug-treated and NaCl-treated cold-exposed groups ($P < 0.05$ in both cases) than in the corresponding guinea pigs kept at room temperature. No differences were seen between the drug-treated animals and their controls (Table 6). There was no detectable adrenaline in the vitreous fluid of any animal.

Liver and muscle glycogen and blood glucose

There were no differences in liver glycogen concentrations between the cold-exposed drug-treated animals and their controls or between the room temperature animals and their controls. The cold-exposed animals had lower liver glycogen concentrations than those kept at room temperature, but no statistically significant differences were seen between groups. Muscle glycogen concentrations were also lower in the cold-exposed groups, but no statistically significant differences existed. Blood glucose was higher in both chlorpromazine-treated groups than in the control groups, the difference being statistically significant at room temperature ($P < 0.01$), but not in severe cold. The cold-exposed NaCl-treated guinea pigs also had significantly higher blood glucose than the NaCl-treated animals kept at room temperature ($P < 0.05$). The chlorpromazine-treated cold-exposed animals had a significantly higher blood glucose concentration than the room temperature group treated with the drug ($P < 0.01$) (Table 7).

Table 7. Liver and muscle glycogen and blood glucose in guinea pigs kept in severe cold or at room temperature and treated with chlorpromazine (CPZ) 20 mg · kg^{-1} or 0.9% NaCl i.p.

Group		Liver glycogen ($mg \cdot g^{-1}$)	Muscle glycogen ($mg \cdot g^{-1}$)	Blood glucose ($mmol \cdot l^{-1}$)
I	CPZ, −20°C)	7.51 ± 5.65 ($n = 8$)	1.69 ± 1.19 ($n = 8$)	12.63 ± 1.04** ($n = 7$)
II	(NaCl, −20°C)	11.63 ± 13.81 ($n = 8$)	1.55 ± 1.13 ($n = 8$)	10.76 ± 2.20* ($n = 8$)
III	(CPZ, +23°C)	22.83 ± 9.94 ($n = 8$)	2.21 ± 1.64 ($n = 8$)	9.99 ± 1.44*** ($n = 8$)
IV	(NaCl, +23°C)	24.06 ± 21.03 ($n = 8$)	2.46 ± 1.95 ($n = 8$)	7.40 ± 0.68 ($n = 8$)

Values are means ± SD. Statistical significance: * $P < 0.05$ compared to NaCl-treated group kept at +23°C; ** $P < 0.01$ compared to CPZ-treated group kept at +23°C; *** $P < 0.01$ compared to NaCl-treated group kept at +23°C; Bonferroni's test

Autopsy findings

Gastric mucosal bleeding was seen in all except one of the guinea pigs exposed to severe cold for 1 h and treated with chlorpromazine. The corresponding NaCl-treated animals did not have any bleeding. Only one drug-treated animal had gastric mucosal bleeding after 1 h at room temperature, whereas none of their NaCl controls had any bleeding.

Discussion

As expected, the chlorpromazine-treated animals became obviously hypothermic after 1 h at −20°C. The drop in colon temperature in the control animals kept in the cold may at least partly be because of restraint. At room temperature restraint did not cause any alterations in the colon temperature during the experiment, as seen in the constant temperature curve. The hypothermic effect of chlorpromazine could already be seen after 1 h at room temperature, which is in agreement with earlier observations [1, 2].

The relatively high dose of chlorpromazine did not cause any apparent vasodilation at room temperature, although the ear-lobe temperature was slightly higher in the drug-treated group during the first 10–15 min. A very rapid initial vasodilation may have taken place in the drug-treated animals immediately after the i.p. injection, as reported with ethanol in rats [12], but this was not observed here.

The higher hypothalamic MHPG concentrations and lower NA concentrations in both cold-exposed groups, as compared with the animals kept at room temperature, indicate a cold-induced increase in the activity of noradrenergic neurons in this brain region. An elevated MHPG concentration is a useful indicator of increased noradrenergic neuronal activity [13]. The rise in the MHPG/

NA ratio in the hypothalamus, which was clearly observable in the present cold-exposed groups, is also thought to be consistent with increased activity of noradrenergic neurons [14]. Chlorpromazine had no significant effect on the cold-induced activity of noradrenergic neurons in the hypothalamus, as no differences were seen in the hypothalamic NA and MHPG. It is possible, however, that it may have stimulated the extraneuronal metabolic pathway of NA, leading to an accumulation of normetanephrine, as reported in rats by Westerink [5]. In other words, cold increased the release of NA and its metabolism to MHPG, but the NA receptor blockade caused by chlorpromazine may have had an inhibitory effect on this metabolic pathway.

The similar HVA concentrations in the NaCl-treated guinea pigs kepts in the cold and at room temperature can be interpreted as suggesting that the hypothalamic dopaminergic neurons are not activated by cold; this is also true of the similar HVA/DA ratios in these groups. HVA is the main central metabolite of DA, and its concentration in the brain tissue should be an indicator of dopaminergic neuronal activity. A review of the literature on this has been published by Scheinin [10]. The elevated DA concentration in the cold-exposed animals may reflect increased synthesis of this compound, which could be used as a precursor of NA.

Chlorpromazine had no significant effect on hypothalamic DA either at room temperature or in severe cold, but it did increase the amount of HVA. This is in accordance with earlier studies in experimental animals, in which a chlorpromazine-induced increase in cerebrospinal fluid HVA has been reported [15]. This increase is interpreted as implying that the drug-induced DA receptor blockade increases the turnover of DA. The same tendency was seen here in severe cold.

According to the literature review of Scheinin [15], the concentration of 5-HIAA in the cerebrospinal fluid and brain tissue is an indication of the activity of the serotonergic neurons and is not affected by antipsychotic drugs. Increased serotonergic activity in the hypothalamus was seen in the present study in both cold-exposed groups and is probably also reflected by the higher 5-HIAA/5-HT ratios observed after cold exposure. Chlorpromazine did not cause any changes in serotonergic neurotransmission at room temperature, whereas in severe cold hypothalamic 5-HIAA was lower in the drug-treated animals than in the NaCl-treated ones, indicating diminished serotonergic activity in the hypothalamus. On the other hand, this could not be seen in the 5-HIAA/5-HT ratios. The high 5-HIAA concentration indicates, however, that there is an increase in the serotonergic neuronal activity in the hypothalamus, which was inhibited by chlorpromazine.

The observation that serum NA and A were lower in both cold-exposed groups than in the corresponding groups kept at room temperature points to a sympathetic "switch-off" phenomenon [16] or exhaustion of the sympathetic nervous system. The relatively low concentrations of urinary NA and A in the cold-exposed chlorpromazine-treated guinea pigs show that the excretion of these catecholamines was not at a high level at any time during cold exposure. That is probably because of the strong tranquillizing effect of chlorpromazine, which was potentiated by the rapid stunning effect of severe cold. A cold-

induced rise in urinary A was seen in the cold-exposed NaCl group, whereas no notable rise in NA was observed. The high serum glucose concentration in the cold-exposed NaCl-treated animals indicates A-induced glucose mobilization. The drug-treated cold-exposed animals also had high serum glucose in spite of relatively low serum and urinary A values, probably as a consequence of other hormone effects, e.g., increased cortisol or decreased insulin, or else glucose uptake into the cells may have otherwise been reduced. Chlorpromazine seemed to liberate DA from peripheral sources, as urinary excretion was mostly seen only in the drug-treated guinea pigs. The significance of this finding remains obscure.

Gastric mucosal bleeding is a frequent finding in humans dying of hypothermia, and the same kind of bleeding was generally present in the drug-treated cold-exposed group of guinea pigs. Although urinary catecholamines did not show any stress response, the stomach was indicative of severe stress conditions, which remained unaffected by a sedative drug.

The rise in NA and DA in the vitreous fluid also proved to be an indicator of cold stress, as reported earlier by Lapinlampi and Hirvonen [17]. As no drug-induced changes in vitreous fluid catecholamine content were seen in the present work, this fluid could be worth analyzing in forensic cases in order to confirm general cold stress.

In conclusion, the current study indicates that hypothalamic noradrenergic and serotonergic neurons are activated by severe cold and that chlorpromazine has an inhibitory effect on serotonergic neurons in severe cold and may also reduce cold-induced noradrenergic activity. Chlorpromazine increased the turnover of DA in the hypothalamus, and this tendency was also observed in a cold environment. A peripheral release of DA was induced by chlorpromazine. Vitreous fluid catecholamines proved to be a useful indicator of cold stress without any observable drug effects, whereas urinary catecholamines were not elevated because of the strong tranquillizing effect of chlorpromazine combined with an anaesthetizing effect of severe cold.

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References

1. Borbely A, Loepfe-Hinkkanen M (1979) Phenothiazides. In: Lomax P, Schönbaum E (eds) Body temperature. Regulation, drug effects and therapeutic implications. Decker, New York, pp 403–426
2. Clark WG, Clark YL (1981) Changes in body temperature after administration of antipyretics, LSD, Δ^9-THC, CNS depressants and stimulants, hormones, inorganic ions, gases, 2,4-DNP and miscellaneous agents. Neurosci Biobehav Rev 5:2–136
3. Kollias J, Bullard RW (1964) The influence of chlorpromazine on physical and chemical mechanism of temperature regulation in the rat. J Pharmacol Exp Ther 145:373–381
4. Lin MT, Chow CF, Chern YF (1978) The effects of a decarboxylase inhibitor, benserazide, on both thermoregulation and chlorpromazine induced hypothermia in rats. J Pharm Pharmacol 30:759–761
5. Westerink BHC (1984) Determination of normetanephrine, 3,4-dihydroxyphenylethyleneglycol (free and total), and 3-methoxy-4-hydroxyphenylethyleneglycol (free and

total) in rat brain by high-performance liquid chromatography with electrochemical detection and effects of drugs on regional concentrations. J Neurochem 42:934–942
6. Lin MT, Chern YF, Chow CF, Li YP (1979) Effects of brain serotonin alterations on hypothermia produced by chlorpromazine in rats. Pharmacology 18:43–49
7. Dunn AD, File SE (1983) Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. Physiol Behav 31:511–513
8. Hirvonen J, Huttunen P (1982) Increased urinary concentration of catecholamines in hypothermia deaths. J Forensic Sci 27:264–271
9. Taylor RB, Reid R, Kendle KE, Geddes G, Curle PF (1983) Assay procedures for the determination of biogenic amines and their metabolites in the hypothalamus using ion pairing reversed-phase high-performance liquid chromatography. J Chromatogr 277:101–114
10. Scheinin M, Chang WH, Kirk KL, Linnoila M (1983) Simultaneous determination of 3-methoxy-4-hydroxyphenylglycol, 5-hydroxy-indoleacetic acid, and homovanillinic acid in cerebrospinal fluid with high-performance liquid chromatography using electrochemical detection. Anal Biochem 131:246–253
11. Lo S, Russel JC, Taylor AW (1970) Determination of glycogen in small tissue samples. J Appl Physiol 28:234–236
12. Huttunen P, Myers RD (1985) Release of norepinephrine from the rat's hypothalamus perfused with alcohol relation to body temperature. Alcohol 2:683–691
13. Scheinin H, Scheinin M (1985) Repetitive measurement of monoamine metabolite levels in cerebrospinal fluid of conscious rats: Effects of reserpine and haloperidol. Eur J Pharmacol 113:345–351
14. Grunstein HS, Gleeson RM, Smythe GA (1986) Relationship between hypothalamic noradrenergic neuronal activity and serum 3-methoxy-4-hydroxyphenylethyleneglycol in the rat. Life Sci 39:207–213
15. Scheinin M (1984) Determination of monoamine metabolites in cerebrospinal fluid and plasma: liquid chromatographic methods and pharmacological applications. Grafia Oy, Turku, Finland
16. Chernon B, Lake CD, Zaritsky A, Finton CK, Casey L, Rainey TG, Fletcher JR (1983) Sympathetic nervous system "switch off" with severe hypothermia. Crit Care Med 11: 677–680
17. Lapinlampi TO, Hirvonen JI (1986) Catecholamines in the vitreous fluid and urine of guinea-pigs dying of cold and the effect of postmortem freezing and autolysis. J Forensic Sci 31:1357–1365

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