BASIC CENTRAL PHARMACOLOGICAL PROPERTIES OF THIOPHOSPHORIC ACID ALKALOID DERIVATIVES FROM *CHELIDONIUM MAJUS* L.

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Basic central pharmacological properties of thiophosphoric acid alkaloid derivatives from Chelidonium majus L. Z. KLEINROK, E. JAGIEŁŁO-WÓJTOWICZ, B. MATUSZEK, A. CHODKOWSKA. Pol. J. Pharmacol. Pharm. 1992, 44, 227–239.

The effects of thiophosphoric acid alkaloid derivatives from *Chelidonium majus* L. (Ukrain, UKSR-222) on the central nervous system (CNS) of mice and rats was studied. Intraperitoneal (*ip*) administration of Ukrain in doses of 9.5 and 19 mg/kg for mice depressed spontaneous motor activity, decreased body temperature and potentiated the action of hexobarbital. Only in a dose of 19 mg/kg Ukrain produced analgesic action in the hot plate test. It had no protective effect against electroshock or pentetrazol-induced seizures. In rats, *ip* administration of Ukrain in dose of 14 and 28 mg/kg potentiated the action of amphetamine and apomorphine but had no effect on catalepsy induced by haloperidol. Ukrain used in dose 9.5, 14, 19 and 28 mg/kg antagonized the head twitches induced by 5-HTP and hyperthermia-induced by m-CPP. Biochemical studies indicated that Ukrain did not affect the NA and DA concentrations in the whole rats' brain and did not affect the 5-HT and 5-HIAA concentrations in the whole brain of rats. These findings demonstrate that the central action of Ukrain involves the stimulation of the dopaminergic system and the inhibition of the serotoninergic system.

Thiophosphoric acid alkaloid derivatives from *Chelidonium majus* L. (the preparation called Ukrain) (Fig. 1) are immunologically effective and

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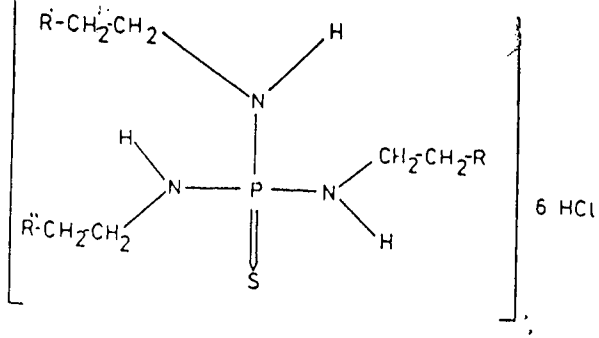

Fig. 1. Structural formula of Ukrain

can improve human cellular response [8, 14, 17]. This semisynthetic drug has unique properties such as selective uptake by human malignant tumor cells and not by normal cells [16]. Furthermore, Ukrain causes a regression of tumors and metastases in many oncological patients [15]. However, the central action of Ukrain has not been yet explained.

Our previous studies [9, 11] showed that chelidonine – the major alkaloid from *Chelidonium majus* L. exerted a depressive action on the central nervous system in rodents. It was found that chelidonine produced an inhibitory effect on the dopaminergic structures in the rat [11].

The main objective of the present work was to study the effects of Ukrain on the central nervous system in mice and rats.

MATERIALS and METHODS

Animals. Male Albino Swiss mice (20–24 g) and Wistar rats (180–200 g) were used throughout the experiments. They were kept in colony cages in a room with controlled temperature (21 ± 1° C). The animals had free access to chow pellets (Murigram, Bacutil, Motycz, Poland) and tap water.

Drugs. Ukrain (thiophosphoric acid alkaloid derivatives from *Chelidonium majus* L.) was synthesized by J. W. Nowicky (Vienna, Austria) [13, 14]. Other reagents used and their sources were as follows: apomorphine hydrochloride, d-amphetamine sulphate, α-methyl-p-thyrosine (α-MT), L-5-hydroxytryptophan (5-HTP) and meta-chlor-phenylpiperazine (m-CPP) were obtained from Sigma Chemical Co., USA., Hexobarbital-sodium (VEB Arzneimittelwerk, Dresden, DDR), pentetrazol (Cardiazolum Polfa, Warszawa, Poland), reserpine (Serpasil, Ciba Geigy, Wehr. Germany). All other chemicals were of analytical grade and were purchased from Merck, Darmstadt, Germany.

The Ukrain was administered intraperitoneally (*ip*) in aqueous solutions, always in the volumes of 0.1 ml/10 g for mice and 0.5 ml/100 g for rats. The control animals received identical volumes of the solvent. In all experiments this alkaloid was used in doses starting from 0.1 LD_{50} and decreasing gradually until there was no further pharmacological activity. Other substances were administered as aqueous solutions, water

The following t[illegible]s were performed:

Determination of acute toxicity. Increasing doses of Ukrain were injected (*ip*) or given orally (*po*) to mice and rats in groups of 6 animals. Acute toxicity after 48 h was assayed as lethal dose (LD_{50}) according to Litchfield and Wilcoxon [12].

Determination of cumulative toxicity (C-LD_{50}). Ukrain was given *ip* to two groups of 5 mice and to two groups of 5 rats starting with a dose of 9% of LD_{50} (for mice or for rats). Those doses were increased by 50% every 4 days so that on days 5–8 the animals received 13.5% of LD_{50} (mice and rats), on days 9–12, 20% of LD_{50} and so on. The experiments were continued until all the mice or rats died, and the C-LD_{50} was calculated according to Thompson [23].

Effect on motor coordination. Rota rod test was carried out on preselected mice or rats which were able to stay on a rotating rod for 2 min. The experiment was carried out according to Gross and Tripod [7]. The animals received the Ukrain and 30, 45, 60, 90 and 120 min later were placed on a rod rotating at the rate of 5 rpm. The number of animals which fell off the rod during this period was counted.

Effect on body temperature of normothermic mice. The rectal temperature was measured with a thermometer (Electrolaboratoriet, Copenhagen) before and after the administration of Ukrain. The experiment was carried out in a room with a temperature of 21 ± 0.5° C.

Effect on spontaneous locomotor activity in mice. The locomotor activity of mice was measured in circular photoresistor actometers (32 cm in diameter). The animals were placed in them 30 min after the injection of Ukrain and motility was recorded for 1 h. Each crossing of the light beam was recorded automatically.

Effect on the action of hexobarbital in mice. Hexobarbital (75 mg/kg *ip*) was administered 30 min after the injection of Ukrain. The sleeping time (from loss to recovery of righting reflex) was measured.

Analgesic effect. a) Acetic acid-induced writhing test [24]. An acetic acid solution (3%) was injected *ip* 30 min after administration of Ukrain. 5 min later, the number of writhing episodes was counted for a 30 min period. *b) Hot plate test* [17]. The mice were placed singly on the hot plate (55 ± 0.5° C) and latency time between heat stimulus application and licking of the paws was measured 15, 30, 45, 60, 90 and 120 min after Ukrain injection.

Convulsive procedure. a) Maximal electroshock was produced, according to Swinyard and al. [22] employing corneal electrodes, alternating current 50 Hz, 50 mA and 0.2 s time of impulse. Mice were given Ukrain 30 min before the test. The presence of a tonic contraction of the extensor muscles as well as the duration of the contraction and mortality were determined. *b) Pentetrazol* (110 mg/kg *sc*) was administered 30 min after the injection of Ukrain. The animals were then placed singly in Plexiglas cages (25 × 15 × 10 cm) and observed for 60 min after the administration of the convulsant drug. The criterion used to indicate convulsive response was either a clonic or tonic seizure, and the number of mice which died during the test period was also taken into consideration.

Effect on the number crossing in the "four plates" test [1]. The test was carried out on mice in a special cage, whose floor consisted of 4 metal plates connected to a DC source. Mice were dropped individually on the cage for a 15 s adaptation period. After that time every crossing from one plate to the other was punished by electric shock (150 V, 0.5 s), which caused a flight reaction. There was a refractory period of 3 s after the shock. The test was carried out for 1 min in which the number of crossings

Effect in the despair test. For acclimatization, 24 h before test session, the mice were forced to swim individually inside vertical glass cylinders containing 15 cm water maintained at 25° C. After swimming 15 min in water, they were removed and allowed to dry for 15 min in a heated enclosure before being returned to their cages. On the test day, 30 min after the Ukrain or placebo injections, they were replaced in the cylinders and the duration of immobility was measured for a 5 min period as described by Porsolt et al. [20, 21].

Effect on amphetamine-induced hyperactivity. Mice were given d-amphetamine (0.5 mg/kg *sc*) 30 min after the administration of Ukrain. Immediately after the injection of amphetamine the mice were placed separately in photocell actometers for 1 h and the counters were read after 30 and 60 min. The control group was tested simultaneously.

Effect on the stereotypy. a) Apomorphine-induced stereotypy. Apomorphine (3 mg/kg *sc*) was administered 30 min after the injection of Ukrain. The rats were placed separately in plexiglass cages. Stereotyped behavior was rated according to a five-point scale similar to that of Janssen et al. [10] and Del Rio and Fuentes [6]: 0 – no stereotypy behavior the same as in the control: 1 – episodic sniffing, explorative activity; 2 – constant sniffing, episodic licking; 3 – constant licking, episodic gnawing; 4 – constant gnawing. The degree of stereotypy was assessed at 15 min intervals over 2.5 h. *b) Amphetamine-induced stereotypy.* Rats were given d-amphetamine (5 mg/kg *sc*) 30 min after the injection of Ukrain. Stereotypy behavior was estimated every 30 min for 4 h using the same scale.

Effect on the apomorphine-induced hypothermia. Mice were given apomorphine (16 mg/kg *sc*) 30 min after administration of Ukrain. The temperature before the injection of Ukrain was accepted as the reference temperature. Body temperature was then measured 30, 60, 90, 120 and 180 min after the injection of Ukrain.

Effect on haloperidol-induced catalepsy. Rats were treated with Ukrain 2 h after administration of haloperidol (1 mg/kg *ip*). Catalepsy was assessed 30 min after Ukrain application and then, every 30 min for 3 h according to the method of Costall and Naylor [4].

Effect on the reserpine-induced hypothermia. Mice were given reserpine (2 mg/kg *sc*) 18 h before the administration of Ukrain. The body temperature was measured rectally by thermistor thermometer. The temperature before the injection of Ukrain was accepted as the reference temperature. Body temperature was measured 30, 60, 90, 120 and 180 min after the administration of Ukrain.

Effect on the head-twitch response with 5-HTP. Groups of 10 animals were used at each dose. Mice and rats were given Ukrain 30 min before 5-HTP (170 mg/kg *ip*). The animals were placed singly into plexiglass cages and observed for 60 min after the injection. The number of head-twitch episodes was counted.

Effect on the m-CPP-induced hyperthermia at high ambient temperature (28° C). Rats were given Ukrain simultaneously with m-CPP (10 mg/kg *ip*). The body temperature was measured as described above. The temperature before the administration of the drugs was accepted as the reference temperature. Body temperature was measured 30, 60, 90, 120, 180 and 240 min after the last injection.

Biochemical investigations

Effect on the level and utilization of noradrenaline (NA) and dopamine (DA) in the whole brain of rats. Ukrain was given 30 min before decapitation. Another group received Ukrain 30 min before α-MT (250 mg/kg *ip*), and the rats were decapitated 2 h after α-MT. The brains were removed and kept at –18° C till the spectrofluorometric assay. The NA and DA concentrations were determined with the method of Chamberlain and utilization of the catecholamines according to Brodie et al. [2] as modified by Papeschi [19].

Effect on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the whole brain of rats. The rats receiving Ukrain were killed 30 min later. 5-HT and 5-HIAA were estimated in the whole rat brain by the spectrofluorometric method of Curzon et al. [5] using a Perkin-Elmer LS-3B spectrofluorometer.

Statistics. The significance of differences between means were calculated using Student's *t* test. P values of 0.05 or less were considered to represent significant differences.

RESULTS and DISCUSSION

The acute toxicity of Ukrain was 190 (174.3–207.1) mg/kg *ip* in mice and LD_{50} was 280 (208.9–375.2) mg/kg *ip* in rats (Tab. 1). Ukrain in near-lethal doses produced sedation, ptosis, tremor and hypothermia. The cumulative median lethal dose (C-LD_{50}) of Ukrain was 86.1% of the LD_{50} value. Intraperitoneal administration of Ukrain in doses of 9.5 and 19 mg/kg to mice and 14 and 28 mg/kg to rats produced no disturbances in motor coordination.

Table 1. Acute toxicity (LD_{50}) of Ukrain after administration in the mice and in the rats

Treatment	Route	LD_{50} (95% confidence limits) mg/kg	
		mice	rats
Ukrain	*ip*	190 (174.3–207.1)	280 (208.9–375.2)
Ukrain	*po*	460 (410.7–515.2)	> 1000

The data are expressed from 5–8 different doses of Ukrain; n = 6/group. For further details see Materials and Methods

The present results suggest that Ukrain markedly affected the central nervous system of rodents producing a depression in spontaneous locomotor activities, caused hypothermia in normothermic mice as well as potentiated the action of hypnotic drug. Ukrain in doses of 9.5 and 19 mg/kg *ip* significantly decreased locomotor activity of mice (Fig. 2), while it was ineffective in a dose of 4.75 mg/kg *ip*. This alkaloid in doses of 9.5 and 19 mg/kg caused in mice hypothermia which lasted for 90 min and reached the value of –1.8° C (Tab. 2).

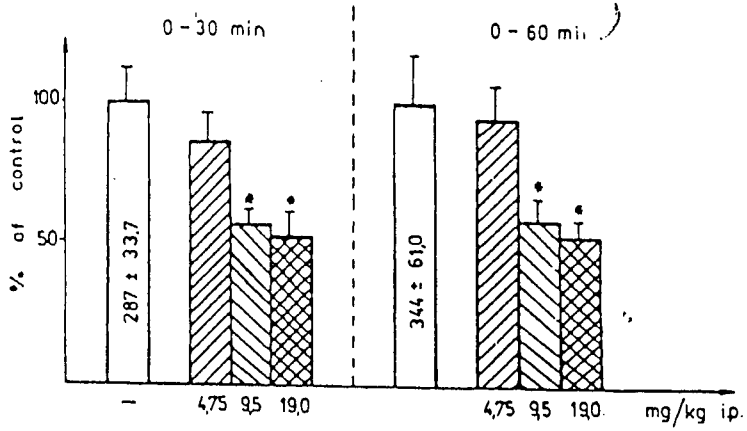

Fig. 2. Effect of Ukrain on the spontaneous locomotor activity in mice. Results are expressed as mean ± SEM; n = 10/group. * – $p < 0.001$ compared with control group

Table 2. Effect of Ukrain on the body temperature of the normothermic mice

Treatment mg/kg *ip*	Basic temperature °C	Time min 30	45	60	90	120
–	36.8 ± 0.1	36.9 ± 0.1	37.0 ± 0.1	37.2 ± 0.1	36.9 ± 0.0	36.9 ± 0.1
Ukrain – 4.75	36.9 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	36.9 ± 0.2	36.8 ± 0.2	36.8 ± 0.1
Ukrain – 9.5	36.6 ± 0.1	35.6 ± 0.2*	35.4 ± 0.2*	35.6 ± 0.1*	36.2 ± 0.1	36.6 ± 0.1
Ukrain – 19.0	36.7 ± 0.1	35.2 ± 0.1*	35.2 ± 0.2*	35.4 ± 0.1*	35.6 ± 0.0*	36.3 ± 0.1

* – $p < 0.001$ compared with control group. Results are expressed as a mean temperature ± SEM; n = 6/group

Ukrain in doses of 2.37, 4.75, 9.5 and 19 mg/kg *ip* significantly prolonged hexobarbital sleeping time (Fig. 3), while it was ineffective at a dose of 1.18 mg/kg. Moreover, the substance tested in doses of 9.5 and 19 mg/kg *ip* decreased hypothermia in reserpinized mice (Tab. 3). This data are in agreement with the results on the sedative action of chelidonine an isoquinoline alkaloid from *Chelidonium majus* [9].

Table 3. Effect of U[illegible]n on reserpine-induced hypothermia in mice

Treatment mg/kg *ip*	Basic temperature °C	Time 30 min	1 h	1.5 h	2 h	3 h
Control	37.0	36.9 ± 0.2	37.0 ± 0.1	37.1 ± 0.3	37.1 ± 0.0	37.2 ± 0.1
Reserpine[1] – 2.0	36.8	35.0 ± 0.1	34.6 ± 0.2	34.6 ± 0.3	34.7 ± 0.2	34.7 ± 0.3
Ukrain – 4.75 + reserpine – 2.0	37.1	35.0 ± 0.2	34.9 ± 0.1	34.7 ± 0.2	34.7 ± 0.2	34.8 ± 0.1
Ukrain – 9.5 + reserpine – 2.0	37.0	36.7 ± 0.1*	36.9 ± 0.0*	37.0 ± 0.2*	36.9 ± 0.2*	36.8 ± 0.2*
Ukrain – 19.0 + reserpine – 2.0	36.9	37.0 ± 0.1*	37.0 ± 0.2*	36.9 ± 0.3*	36.8 ± 0.2*	36.9 ± 0.1*

Results are expressed as a mean ± SEM. [1]Reserpine was given *sc* to mice 18 h before injection of Ukrain. * – $p < 0.001$ compared with reserpine group; n = 6/group

Our results indicate that Ukrain in a dose of 19 mg/kg *ip* produced analgesic action in the hot-plate test (Tab. 4), but did not influence the nociceptive response in the "writhing test".

Table 4. Antinociceptive effect of Ukrain administration *ip* in mice. Results are expressed as a mean percent of control latency time ± SEM, n = 10, * – $p < 0.001$ compared with control

Substance mg/kg *ip*	Time min 15	30	45	60	90
Ukrain – 9.5	119 ± 8.9	120 ± 8.8	118 ± 12.8	122 ± 13.5	120 ± 11.8
Ukrain – 19.0	151 ± 13.4*	143 ± 14.7*	140 ± 11.4*	114 ± 8.1	96 ± 10.5

Our observations show that Ukrain did not possess either anxiolytic or anticonvulsive properties, but enhances the stimulatory effects of dopaminergic stimulants, amphetamine and apomorphine. Ukrain in doses of 9.5 and 19 mg/kg *ip* significantly increased the amphetamine-induced locomotor hyperactivity recorded after 30 and 60 min (Fig. 4) and in

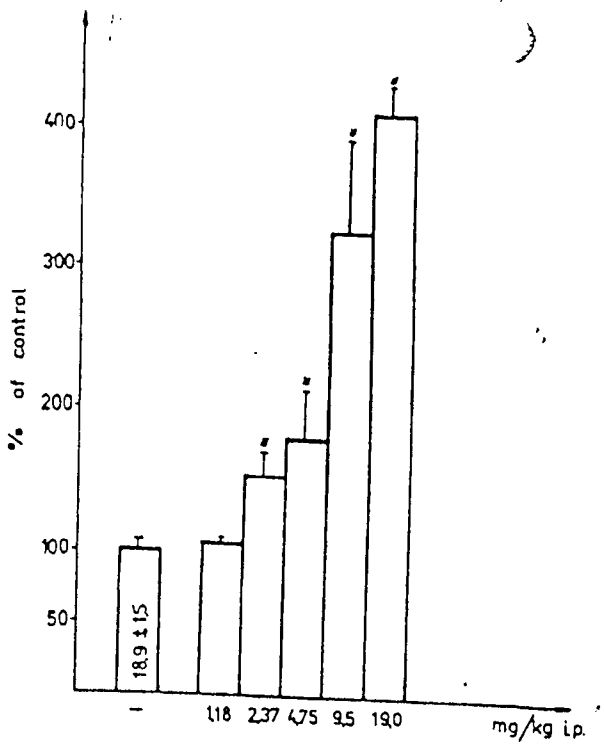

Fig. 3. Effect of Ukrain on time of hexobarbital sleep in mice. Results are expressed as mean ± SEM; n = 8/group. * – $p < 0.001$ compared with control group

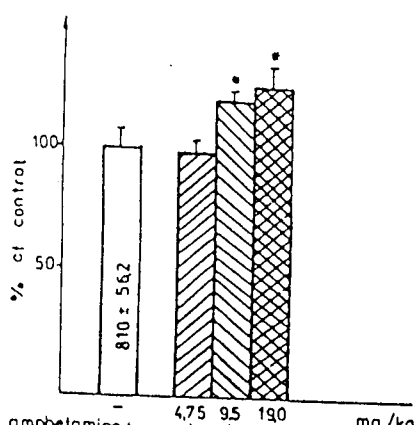

Fig. 4. Effect of Ukrain on the d-amphetamine-induced hyperactivity in mice. Amphetamine was administered 30 min after the injection of Ukrain. Locomotor activity measurements were done immediately after the last injection for 1 h. * – $p < 0.001$ compared with amphetamine group; n = 10/group

doses of 14 an 28 mg/kg *ip* increased the intensity and incidence of amphetamine- or apomorphine-induced stereotypy in rats (Fig. 5). Ukrain in doses of 9.5 and 19 mg/kg *ip* significantly enhanced the apomorphine-induced hypothermia in mice (Fig. 6), but did not affect haloperidol-induced catalepsy. No characteristic changes were found in the concentrations of NA and DA in the whole rats brain, but the increased utilization of both amines was shown (Fig. 7). Our previous study [9, 10] demon-

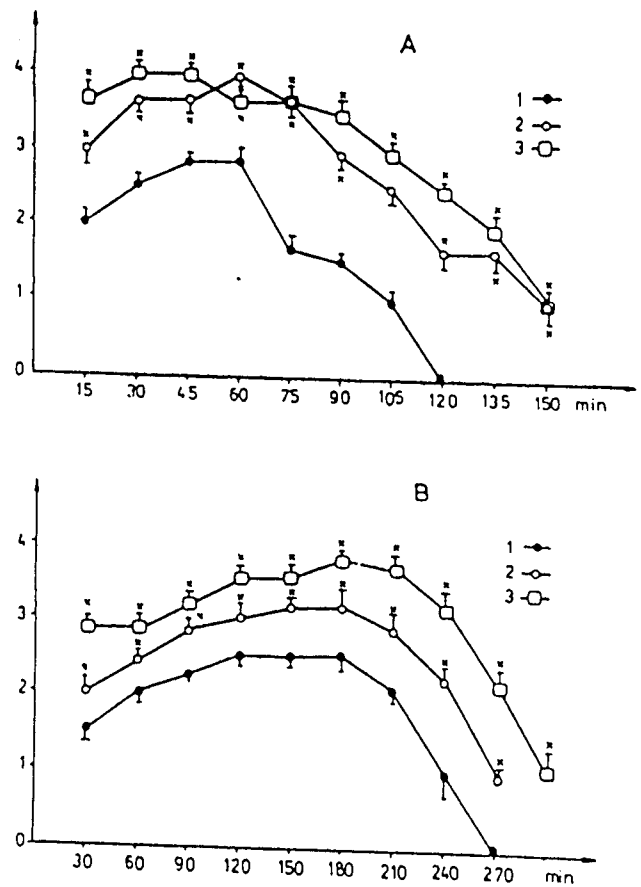

Fig. 5. Effect of Ukrain on apomorphine (A) or amphetamine (B) stereotypy in rats. Ukrain was administered 30 min before the apomorphine or amphetamine. Stereotypy behavior was estimated according to 5 point scale (as described in Meterials and Methods) at 15 min intervals during 2.5 h (for A) and 30 min intervals during 4 h (for B). Explanations for A: 1 – apomorphine (3 mg/kg, *sc*), 2 – Ukrain (14 mg/kg) + apomorphine, 3 – Ukrain (28 mg/kg) + apomorphine. Explanations for B: 1 – d-amphetamine (5 mg/kg, *sc*), 2 – Ukrain (14 mg/kg) + amphetamine, 3 – Ukrain (28 mg/kg) + amphetamine. Results are expressed as mean ± SEM; n = 8/group. * – $p < 0.001$ compared with apomorphine (for A) or amphetamine (for B)

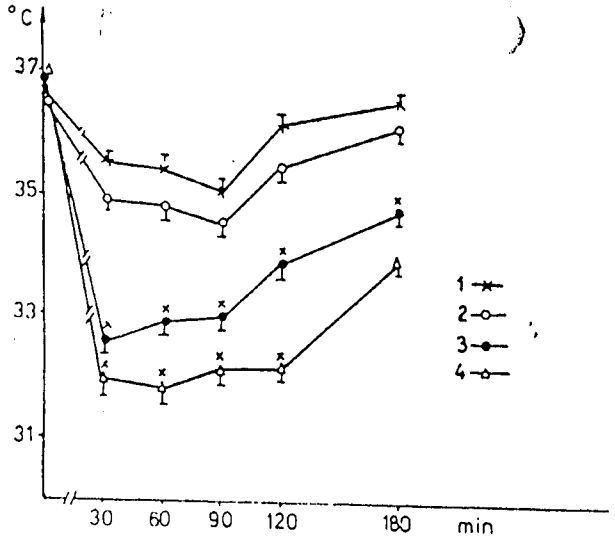

Fig. 6. Effect of Ukrain on apomorphine-induced hypothermia in mice. Explanations: 1 – apomorphine (16 mg/kg *sc*), 2 – Ukrain (4.75 mg/kg).+ apomorphine, 3 – Ukrain (9.5 mg/kg) + apomorphine, 4 – Ukrain (19 mg/kg) + apomorphine. Results are expressed as mean ± SEM; n = 6/group. * – $p < 0.001$ compared with control group

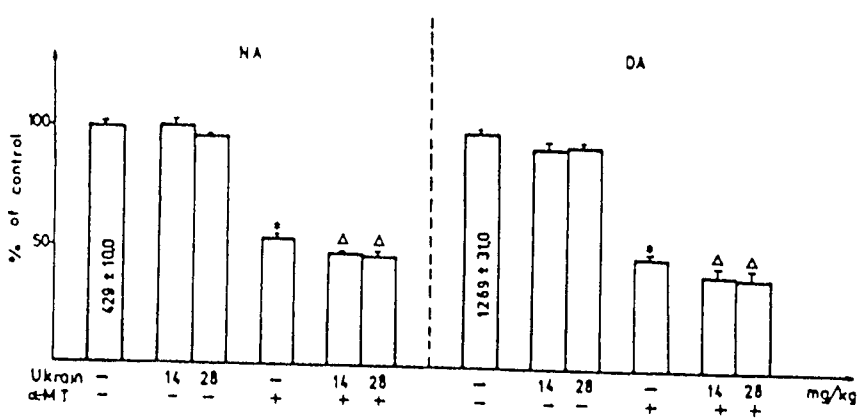

Fig. 7. The levels of noradrenaline (NA) and dopamine (DA) in whole brain rats after treatment with Ukrain and α-methyl-p-tyrosine (α-MT). Results are expressed as mean ± SEM. * – $p < 0.05$ as compared to control group. Δ – $p < 0.05$ as compared to α-MT treated rats; n = 10/group

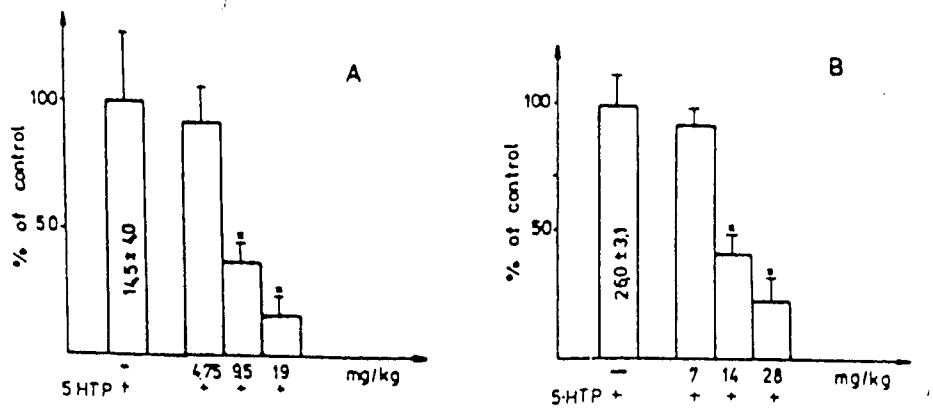

Fig. 8. Effect of Ukrain on the head twitches response induced by 5-hydroxytryptophan (5-HTP) in mice (A) and rats (B). Results are expressed as mean ± SEM. * – $p < 0.001$ compared with control group; n = 10/group. Head twitches were counted in individual animals for 60 min after administration 5-HTP. Ukrain was injected 30 min before 5-HTP

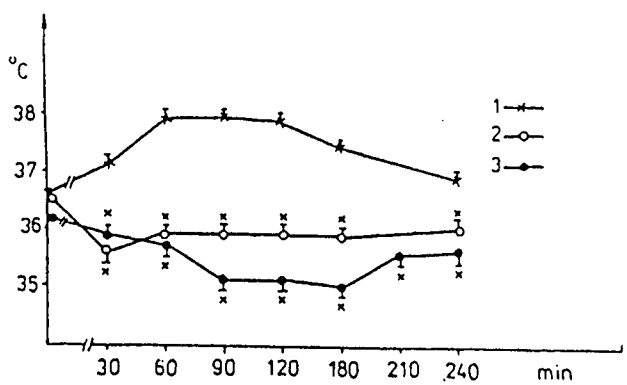

Fig. 9. Effect of Ukrain on the metachlorphenylpiperazine (m-CPP) induced hyperthermia in rats. Explanations: 1 – control group, 2 – Ukrain 14 mg/kg, 3 – Ukrain 28 mg/kg. Results are expressed as mean ± SEM. * – $p < 0.001$ compared with control group; n = 8/group

strated that chelidonine exerted a depressive action on dopaminergic transmission. It was reported that chelidonine decreased amphetamine- and apomorphine-induced hyperactivity and inhibited stereotypy induced by dopaminergic drugs. Besides, chelidonine significantly inhibited the yawning and penile erection produced by apomorphine. However, *ip* administration of chelidonine depressed the whole brain DA concentration and enhanced DA utilization.

The present results indicate that Ukrain may ha[illegible]nterfered with serotoninergic structures. Ukrain in doses of 9.5 and 19 mg/kg *ip* (for mice) or 14 and 28 mg/kg *ip* (for rats) was found to decrease the number of head twitches induced by 5-HTP (Fig. 8). The depressive effect of Ukrain on the serotoninergic system is indicated by the decrease in hyperthermia induced by m-CPP (Fig. 9). However, in doses of 14 and 28 mg/kg *ip* Ukrain practically did not affect the 5-HT and 5-HIAA concentration in the whole rat brain. Similar effects were produced by chelidonine [9].

In conclusion, the present study shows that the central action of Ukrain involves the stimulation of the dopaminergic system and the inhibition of the serotoninergic system. These results partially explain the mechanism of side-effects observed during therapy with Ukrain.

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