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About the Mechanism of the Analgesic Activity of Highly Efficient Analgesics of the New Structural Type: *in vitro* Experiments

E. A. MOROZOVA[1], T. A. ZAPARA[2], A. C. RATUSHNYAK[2], S. O. VECHKALOVA[2], T. G. TOLSTIKOVA[1] and E. E. SHULTS[1]

[1]*Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Pr. Akademika Lavrentyeva 9, Novosibirsk 630090 (Russia)*

E-mail: morozova@nioch.nsc.ru

[2]*Design and Technological Institute of Computer Engineering, Siberian Branch, Russian Academy of Sciences, Ul. Rzhanova 6, Novosibirsk 630090 (Russia)*

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Abstract

The effect of pyrrolidinomorphinane derivatives possessing an analgesic activity on the membrane potential of isolated neurons of a mollusc treated with selective antagonists of opioid receptors was studied. The results confirm the opioid mechanism of the action of these compounds shown earlier in experiments *in vivo* on mice and indicate the nonselectivity of the interaction of the agent T-11 with all types of opioid receptors, as well the preference of the interaction of the agent Sh-42 with κ-receptors.

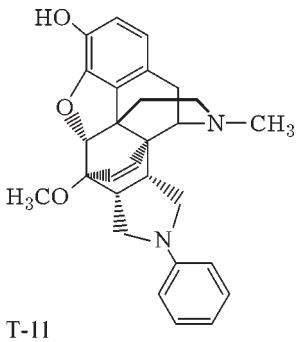
Key words: pyrrolidinomorphinanes, membrane potential, isolated neurons, opioid receptors

INTRODUCTION

With the progress of medicinal chemistry, when creating medical preparations, the chemical modification of plant metabolites is efficiently applied today. In the course of an active collaboration of chemists and pharmacists, it was established that synthetic transformations of molecules of biologically active vegetable substances facilitated the enhancement of their natural activity, decreased undesirable side effects and revealed absolutely new useful properties. In this regard, it is impossible to ignore the constant interest to the substances obtained by the way of chemical transformations of thebaine and the accumulated rather impressive library of the compounds synthesized on its base [1].

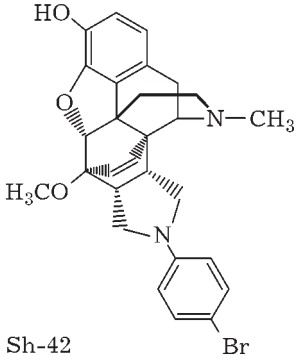
Among them, 6,14-endoetheno-7,8-pyrrolidinomorphinane derivatives – compounds showing a high analgesic activity in various tests on the visceral and thermal experimental pain [2–4], deserve an attention. Out of 26 investigated compounds of this group, two most active agents (compounds T-11 and Sh-42) that differ by the presence of bromine atoms only, have been chosen for a further thorough study (Scheme 1).

The presence of a bromine atom in the structure of the agent Sh-42 seems to have defined the selectivity of its analgesic action. It was established that this compound showed the effect only in tests on the visceral pain, *viz*., acetic cramps (AC) and acetylcholine cramps (AchC), while the agent T-11 displays a high analgesic activity in tests caused by the thermal irritation, *viz*., hot plate (HP) and tail retraction (TR) (Table 1). In addition, it was discovered that the analgesic activity of these agents in AC test was partially lifted (by 45 and 22 % for



T-11

7α, 8α-[N′-(phenyl)-pyrrolidino]-6,14-endoetheno-6,7,8,14-tetrahydrooripavine



Sh-42

7α, 8α-[N′-(4-bromophenyl)-pyrrolidino]-6,14-endoetheno-6,7,8,14-tetrahydrooripavine

Scheme 1.

the agents T-11 and Sh-42, respectively), by the introduction of naloxone 10 min prior to the reproduction of the model. This evidences the involvement of the opioid system into the mechanism of the analgesia of compounds studied [5].

However, the question about the participation of the specific type of opioid receptors (OR) remains open. The goal of this work is the study of the interaction of the agents T-11 and Sh-42 with various types of OR *in vitro* experiments.

EXPERIMENTAL

Experiments were carried out on isolated neurons from peripharyngeal ganglia of the clam *Lymnaea stagnalis* (n = 126). Neurons were isolated by the mechanical defragmentation of ganglia after the treatment with the 0.3–0.5 % solution of pronase (Protease type XIV, Sigma, the USA). Isolated cells were placed on glass substrates that contained the physiological solution of the following composition (μmol/L): NaCl 55, KCl 1.6, $CaCl_2$ 4, $MgCl_2$ 1.5, $NaHCO_3$ 10; pH 7.6–7.8, in a Petri dish. The registration of the electrical activity was carried in 18–20 h after the isolation of cells at room temperature. The microelectrode technique of studies was used.

Concentrations of agents were selected in preliminary experiments according to their effect on the membrane potential (MP) of neurons (μmol/L): T-11 – 0.86, Sh-42 – 1.4. The agents (20 μL) were applied using an automatic micropipette closely situated to a neuron. Antagonists (Sigma, the USA) were added directly into a Petri dish (the volume is 5 mL) in the following concentrations: antagonist μ-OR, H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP) – 1 μmol/L; κ-OR, nor-binaltorphimine and δ-OR Naltrindole hydrochloride – 10 μmol/L. The effect of blockers of OR was evaluated by of the ability of cells to demonstrate a typical reaction on the agents T-11 and Sh-42 after their preliminary incubation with antagonists.

TABLE 1

Analgesic activity of the agents T-11 and Sh-42 on various molecules

Agents	Number of cramps Latent time, s			
	Acetic cramps	Acetylcholine cramps	Hot plate	Tail retraction
T-11	0.0±0.0*	0.0±0.0*	26.5±3.1*	31.4±1.7*
Sh-42	0.0±0.0*	0.0±0.0*	12.0±1.4	9.2±0.4
Control	12.4±0.8	1.5±0.2	9.4±1.0	8.4±0.3

* $p < 0.05$ relatively to the control.

RESULTS AND DISCUSSION

Effect of the agents T-11 and Sh-42 on the membrane potential of intact neurons

Results of the conducted experiments showed that applications of the tested agents T-11 and Sh-42 in concentrations at the level of μmol/L caused direct reactions of neurons. These changes of MP stipulated by the action of the substances were observed during 30–40 min. During the same time, the resting potential (RP) of neurons treated with the agents T-11 or Sh-42 went over to a new level and got stabilized (Fig. 1).

The application of the agent T-11 for all neurons investigated caused a long-lived hyperpolarisation that on average did not exceed (6±2) mV. Further, a partial reduction of MP and the stabilization of RP on a more negative level, in comparison with RP of intact neurons was observed (see Fig. 1). The agent T-11 displayed a high analgesic activity in all conducted *in vivo* tests had a hyperpolarising effect on the neurons of molluscs. The analgesic activity of the known agonists μ-, δ- and κ-OR for many types of cells is connected with the hyperpolarisation as a result of the conductivity increase of the membrane for K^+ [6–8].

The agent Sh-42 had the opposite effect on MP of neurons. The application of this agent caused a depolarisation shift of MP of neurons having a two-phase character. In the beginning, a short-time (6.5–7 min) depolarisation shift of MP evolved, as a result of which, its value approached to the basic level of RP. Then, the second rise of MP is noted that does not exceed the first one by the amplitude, however, it is more stable and preserved during the whole observation time.

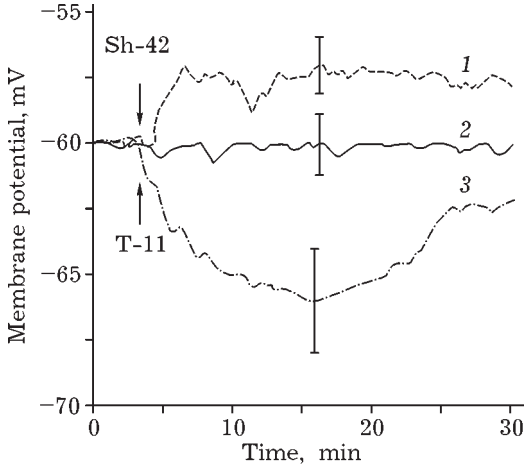


Fig. 1. Effect of the agents T-11 and Sh-42 on the membrane potential (MP) of neurons: *1* – changes of MP caused by the application of the agent Sh-42 (n = 20); *2* – the resting potential of intact neurons (n = 10); *3* – changes of MP caused by the application of the agent T-11 (n = 12).

Possibly, the depolarisation shift of MP caused by the concentrations of Sh-42 at the level of μmol/L is conditioned by its membranotropic action. It is shown for neurons of a pond snail that the nonselective antagonist of μ-, δ- and κ-OR tramadol, agonist κ- and antagonist μ-OR butorphanol, agonists μ-OR promedol and morphine suppress sodium, calcium and potassium ionic currents [9]. At the blockade of sodium channels by OR ligands a membrane of cells is depolarised what is reflected in functioning neurons. According to data of works [10], the membrane-tropic action of opioid analgesics is similar to the effect of local anesthetics on neurons. However, it is impossible to exclude and the direct impact of Sh-42 on OR. The nervous system of molluscs possesses a developed opioid system that includes both specific OR and endogenous peptides and does not have the principal differences from the opioid system of higher animals [11]. It was shown on various types of neurons of mammals that ligands of opioids, depending on the concentration and type of cells could render double hyper- and depolarisation effects with a diverse time dynamics. Low concentrations of the agonists μ-, δ- and κ-OR (lower than nmol/L) cause a short-time increase of the conductivity of a membrane for K^+ (within 1 min), followed by a long-time conductivity decrease accompanied by depolarisation. The action of higher concentrations (at the level of μmol/L), on the contrary, leads to a fast and long increase of the conductivity of a membrane for K^+ and hyperpolarisation [12]. However, some agonists κ-OR with concentrations at the level of μmol/L cause the depolarisation of the membrane of cells, what may be due to the decrease of the membrane conductivity for potassium ions mediated through the system of G proteins and intracellular mediators [13, 14]. Thus, the agent Sh-42, by the action on

TABLE 2

Retention of the reaction of neurons on the introduction of the agents T-11 and Sh-42 on the background of preincubation of the cells with antagonists of OR (I phase of the reaction of neurons on Sh-42 is fast, II phase is long-time)

Antagonists	Agent		
	T-11	Sh-42	
CTAP (μ-antagonist)	100 % (6 out of 6)	I phase	100 % (6 out of 6)
		II phase	100 % (6 out of 6)
Norbinaltorphimine (κ-antagonist)	100 % (6 out of 6)	–	
Naltrindole hydrochloride (δ-antagonist)	100 % (6 out of 6)	–	
μ-antagonist + κ-antagonist	86 % (18 out of 21)	I phase	100 % (out of 12)
		II phase	0 % (out of 12)
μ-antagonist + κ-antagonist + δ-antagonist	86 % (18 out of 21)	I phase	100 % (out of 6)
		II phase	0 % (out of 6)

MP, displays itself analogously to the agonists κ-OR, while the identified selectivity of the analgesic action in tests on the visceral pain indicates its preferable interaction with κ-OR.

Influence of agents T-11 and Sh-42 on MP of neurons, treated with antagonists of opioid receptors

The preincubation of neurons with selective antagonists of various types of OR (μ-, δ- and κ-) showed that none of these antagonists of OR did not block independently reactions of neurons caused by the agent T-11 (Table 2). These data agree with a high analgesic activity of the agent T-11 discovered *in vivo* tests and confirm the suggestion that T-11 can act as a nonselective agonist of μ-, δ- and κ-OR (see Table 1). The simultaneous preincubation of neurons with the antagonists μ- and κ–OR blocked the reactions of cells on application of the agent T-11 in 86 % cases (18/21). The combination from three antagonists did not increase the number of cells that do not respond to the application of T-11. These data can also indicate the membrane-tropic action of the agent associated with the suppression of the ionic conductivity of neurons membrane.

The application of the agent Sh-42 caused a two-phase low-amplitude depolarisation shift of the neuron MP, as established above (see Fig. 1). The preincubation of neurons with selective antagonists of three types of OR showed that none of these antagonists of OR did not block the fast phase of depolarization that was caused by the application of Sh-42. After the preincubation of neurons with norbinaltorfimine (κ-receptor antagonist) the agent Sh-42 did not cause the late reaction phase, viz, the stable depolarisation shift of RP. The first phase of the depolarisation shift is conditioned by the membranotropic action of the agent Sh-42. Possibly, this is associated with the ionic conductivity suppression of a neurons membrane (sodium, calcium and potassium). A more slowly developing stable depolarisation is suppressed by the antagonist κ-OR, which indicates the direct action of Sh-42 on this type of OR.

CONCLUSION

Thus, the experiments carried out on isolated neurons of a mollusc confirm the opioid mechanism of action of highly effective analgesics of the pyrrolidinomorphinane row found earlier *in vivo* experiments on mouse. Besides, results of this work indicate the nonselectivity of the interaction of the agent T-11 with all types of OR and preference of the interaction of the agent Sh-42 with κ-OR.

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