EFFECT OF VARIOUS INDOLYLALKYLAMINES ON THE MOTOR CELLS OF MOLLUSK EMBRYOS AND THE BLOOD VESSELS OF THE RABBIT EAR

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Serotonin is one of the most widespread physiologically active substances, and structures sensitive to it can be detected in the most varied animals, including preneural organisms [1, 3, 5, 7]. However, the question of the specific peculiarities of these receptors in different groups of animals remains to a considerable degree unresolved. It is known only that the antiserotonin effect of the diethylamide of lysergic acid, established in experiments on vertebrate animals, is not always detected in invertebrates [8]. It has also been shown that the myocardium of the Roman snail and organs of vertebrates sensitive to serotonin react similarly to various serotonin analogs [4].

A more detailed comparative study of receptors sensitive to serotonin is of undoubted interest in connection with the problem of the phylogenetic origin of the regulatory mechanisms. In particular, a comparison of the properties of serotonin-sensitive structures in innervated and noninnervated organs of various animals is of special interest. For this purpose, in our experiments we investigated the influence of various indolylalkylamines on the motor properties of developing embryos of nudibrachiates and on the tone of the blood vessels of the rabbit ear.

EXPERIMENTAL PROCEDURE

The experiments on nudibrachiates were conducted at the Murmansk Marine Biological Institute. The main objects of this series of experiments were embryos of Dendronotus frondosus, at various stages of development—from the emergence of the embryonic motor system to the moment of hatching from the egg. It had been shown earlier that the motor cells of embryos of nudibrachiates are very sensitive to serotonin. This property arises long before the appearance of a nervous system, increases for some time, and decreases with the establishment of innervation of the motor cells [1]. Equal numbers of embryos of the same age were transferred to solutions of the test substances, prepared in sea water. The rate of rotation of the embryos at 12-14°, 10 min after the introduction of the test substance, served as the measure of the activity of the preparations. In the control experiments, embryos were placed in filtered sea water. The vasoconstrictive action of indolylalkylamines was studied on an isolated rabbit ear. Solutions of the preparations were made up in Kravkov's fluid. The relative decrease in the number of drops of effluent liquid in comparison with the initial level, taken as 100, served as the measure of activity of the preparations.

In the experiments we used tryptamine and its derivatives, the indole rings of which contain substituents in the form of halogen, methyl, hydroxy, or alkoxy groups. In addition, we studied tryptamine derivatives with several substituent groups or with substituents in the aminoethyl chain, and with lengthening of the latter by one or two carbon atoms. β-3-Indazolylethylamine, structurally close to tryptamine (β-3-indolylethylamine), was also used. Hydroxy derivatives of tryptamine were used in the form of their creatin sulfate complexes, and the remaining substances in the form of their hydrochlorides. A total of 31 compounds were investigated.

EXPERIMENTAL RESULTS

The results of experiments on embryos of D. frondosus are presented in Figs. 1-3. It was found that all the indolylalkylamines tested possessed the ability to stimulate the embryonic motor system to one degree or another. Sensitivity to the initial compound—tryptamine—did not remain constant during development, reaching the highest

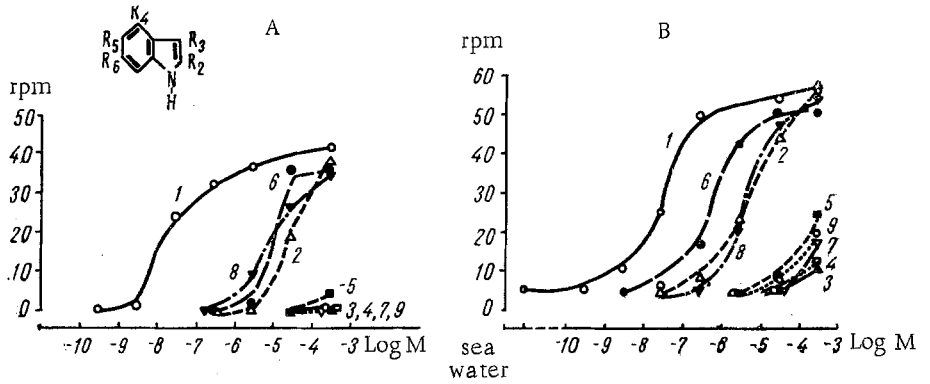

Fig. 1. Effect of various indolylalkylamines on the embryonic motor system of D. frondosus. A) Very early veligers; B) early veligers; 1) serotonin; 2) tryptamine; 3) α-methyltryptamine; 4) 2-methyltryptamine; 5) 4-methyltryptamine; 6) 5-methyltryptamine; 7) 6-methyltryptamine; 8) N,N-dimethyltryptamine; 9) δ-3-indolylbutylamine. Along X-axis—logarithm of the molar concentration of indolylalkylamines; along Y-axis—rate of rotation of the embryos.

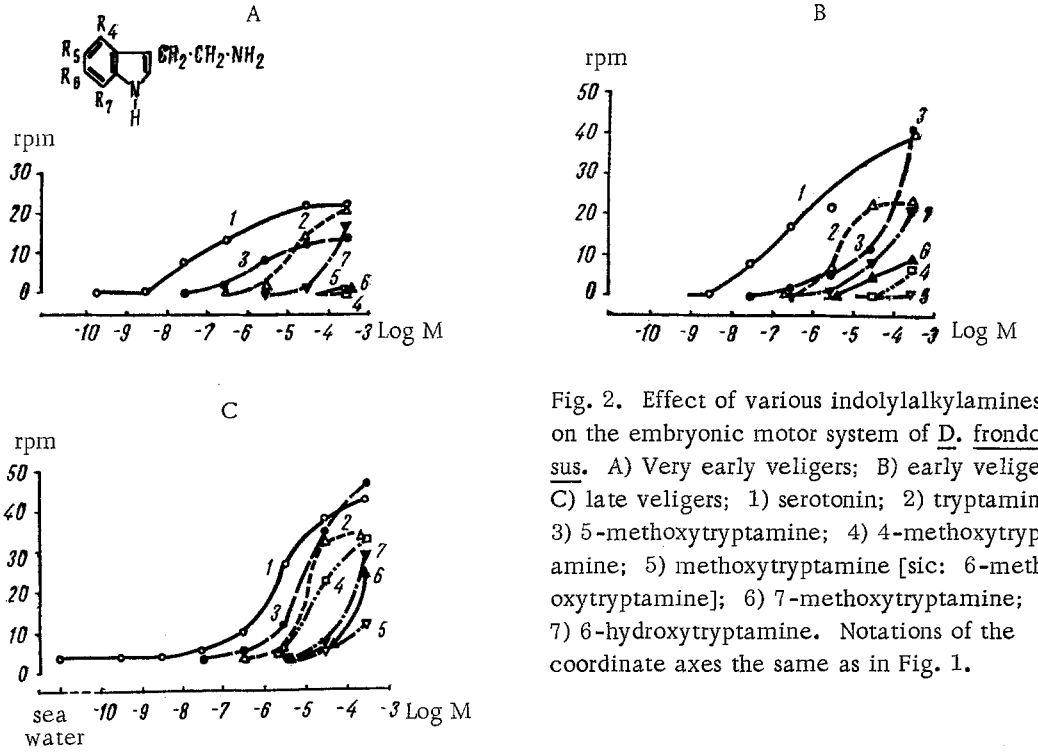

Fig. 2. Effect of various indolylalkylamines on the embryonic motor system of D. frondosus. A) Very early veligers; B) early veligers; C) late veligers; 1) serotonin; 2) tryptamine; 3) 5-methoxytryptamine; 4) 4-methoxytryptamine; 5) methoxytryptamine [sic: 6-methoxytryptamine]; 6) 7-methoxytryptamine; 7) 6-hydroxytryptamine. Notations of the coordinate axes the same as in Fig. 1.

development in medium veligers, and remaining approximately one order of magnitude lower in early and late veligers* (see Figs. 1-3). The introduction of a substituent into the 5-position increased the activity of the

* Veligers are typical mollusk larvae, possessing a characteristic organ of locomotion—the velum, or parus. In very early veligers, the velum is almost undeveloped, while in early veligers it is weakly developed, but the embryonic shell is absent. In medium veligers, the shell is small, the flagella of the velum are well developed, but still have no innervation. In late veligers, the shell reaches the maximum size, and the motor cells of the velum are innervated.

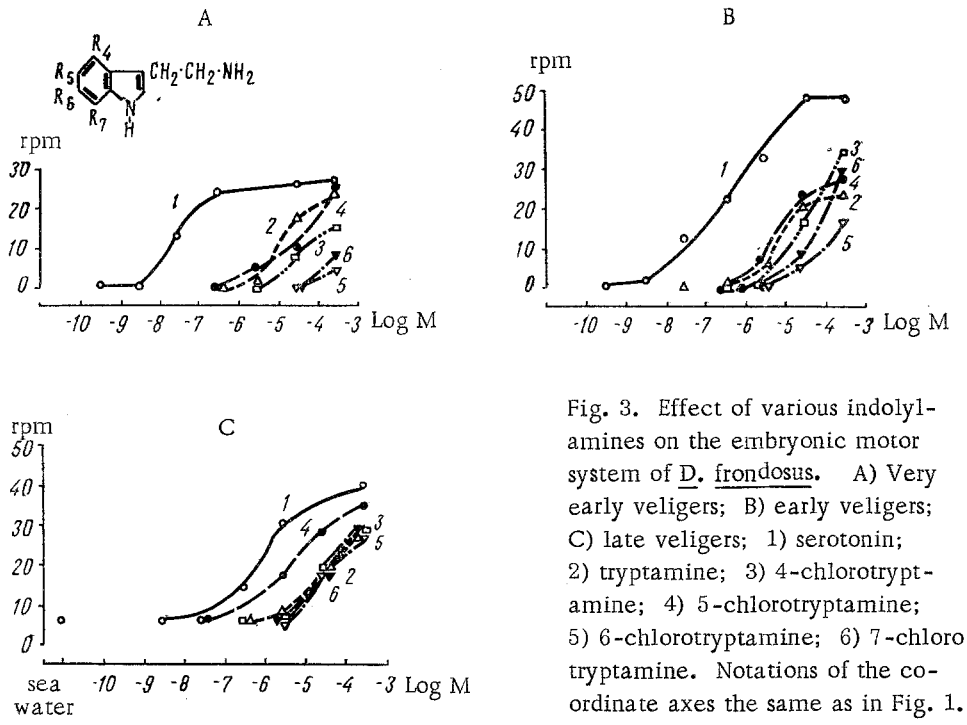

Fig. 3. Effect of various indolylamines on the embryonic motor system of D. frondosus. A) Very early veligers; B) early veligers; C) late veligers; 1) serotonin; 2) tryptamine; 3) 4-chlorotryptamine; 4) 5-chlorotryptamine; 5) 6-chlorotryptamine; 6) 7-chlorotryptamine. Notations of the coordinate axes the same as in Fig. 1.

compounds. Moreover, 5-hydroxytryptamine (serotonin) is the most active of the indolylalkylamines that we investigated. This difference of serotonin from the other indolylalkylamines is most pronounced when it acts on early embryos (Figs. 1A, 2A, 3A). At the late stages of development, this difference is somewhat smoothed out as a result of a decrease in the sensitivity of the embryos to serotonin in connection with the appearance of innervation [1] and an increase in the sensitivity to other indolylalkylamines.

The presence of a substituent in the 2-, 4-, 6-, and 7-positions of the indole ring reduces the activity of the compound in comparison with tryptamine. The activity is also reduced when the side chain is lengthened (δ-3-indolylbutylamine) or with the incorporation of a methyl group in the α-position. This decrease is most pronounced with respect to the early veligers, when certain indolylalkylamines (4-, 6-, or 7-methoxytryptamine, 2-, or 6-methyltryptamine, etc.) prove entirely inactive in the concentrations used (see Figs. 1-3). It is interesting that although the sensitivity to serotonin and tryptamine falls when innervation is established, for all the other indolylalkylamines investigated, on the contrary, it increases noticeably (see Figs. 1-3).

The introduction of supplementary substituting groups into the 5-methoxytryptamine molecule (5-methoxy-7-chlorotryptamine, 1-benzyl-2-methyl-5-methoxy-tryptamine, 5-methoxy-2-carboxytryptamine) sharply reduces the activity, so that it proves even lower than in tryptamine. On the other hand, the replacement of the hydrogen atoms of the primary amino group of tryptamine by methyl groups has no effect upon the activity of the preparation (see Fig. 1). The replacement of carbon in the 2-position of the indole ring by nitrogen (β-3-indazolylethylamine) lowers the activity.

As can be seen from the data presented in the table, similar patterns are also detected in a study of the action of indolylalkylamines on the tone of the blood vessels of the isolated rabbit ear. Tryptamine, its derivatives and homologs reduce the number of drops of effluent liquid, although to varying degrees, i.e., exert a vasoconstrictive action. It is more pronounced in tryptamine derivatives possessing various substituents in the 5-position of the indole ring, where 5-hydroxytryptamine is the most effective preparation. On the contrary, all amines with substituted 2-, 4-, 6-, or 7-position of the indole ring, the α-position of the aminoethyl chain, as well as tryptamine homologs with a lengthened side chain are inferior to the initial compound—tryptamine—in their ability to produce a vasoconstrictive effect. This effect is also appreciably weakened when the molecules of such active preparations as 5-chloro- or 5-fluorotryptamine possess a supplementary substituent in the α-position of the side chain or when the carbon in the 2-position of the indole ring is replaced by nitrogen (β-3-indazolylethylamine). The vasoconstrictive properties of tryptamine were unchanged only when the hydrogens of the primary amino group were replaced by methyls (N,N-dimethyltryptamine).

Vasoconstrictive Effect of Indolylalkylamines in Experiments on Isolated Rabbit Ear

Names of preparations	Concentration (in M)	Decrease in no. of drops (in % of initial)	Names of preparations	Concentration (in M)	Decrease in no. of drops (in % of initial)
Tryptamine	$1.33 \cdot 10^{-7}$	42	Tryptamine	$4 \cdot 10^{-7}$	61
5-Hydroxytryptamine	$1.33 \cdot 10^{-7}$	70	α-Methyltryptamine	$4 \cdot 10^{-7}$	32
5-Methoxytryptamine	$1.33 \cdot 10^{-7}$	62	2-Methyltryptamine	$4 \cdot 10^{-7}$	13
5-Ethoxytryptamine	$1.33 \cdot 10^{-7}$	52	4-Chlorotryptamine	$4 \cdot 10^{-7}$	31
5-Propoxytryptamine	$1.33 \cdot 10^{-7}$	56	4-Methoxytryptamine	$4 \cdot 10^{-7}$	15
5-Butoxytryptamine	$1.33 \cdot 10^{-7}$	58	6-Chlorotryptamine	$4 \cdot 10^{-7}$	24
5-Bromotryptamine	$1.33 \cdot 10^{-7}$	60	6-Methoxytryptamine	$4 \cdot 10^{-7}$	19
5-Iodotryptamine	$1.33 \cdot 10^{-7}$	59	6-Hydroxytryptamine	$4 \cdot 10^{-7}$	34
5-Chlorotryptamine	$1.33 \cdot 10^{-7}$	62	7-Chlorotryptamine	$4 \cdot 10^{-7}$	25
α-Methyl-5-chlorotryptamine	$1.33 \cdot 10^{-7}$	18	7-Methoxytryptamine	$4 \cdot 10^{-7}$	16
5-Fluorotryptamine	$1.33 \cdot 10^{-7}$	62	N,N-Dimethyltryptamine	$4 \cdot 10^{-7}$	64
α-Methyl-5-fluorotryptamine	$1.33 \cdot 10^{-7}$	18	γ-3-Indolylpropylamine	$4 \cdot 10^{-7}$	15
β-3-Indazolylethylamine	$1.33 \cdot 10^{-7}$	31	δ-3-Indolylbutylamine	$4 \cdot 10^{-7}$	14

Thus, serotonin-sensitive receptors of the blood vessels of the rabbit ear and innervated or noninnervated motor cells of mollusk embryos react basically identically to changes in the chemical structure of indolylalkylamines. If we take the sensitivity of these receptors to tryptamine as a basis, then the following may be observed in connection with changes in the chemical structure: a) an intensification of sensitivity—when the 5-position in the indole ring is substituted; b) its conservation at the same level—when the hydrogen atoms of the primary amino group are replaced by methyls; c) weakening of sensitivity—when substituents are introduced into the 2-, 4-, 6-, and 7-positions of the indole ring or into the carbon portion of the aminoethyl chain. Such a dependence of the activity on the structure was also detected earlier [2] in a study of the radio-protective properties of indolylalkylamines.

The data obtained are apparently evidence of the presence of common features in the properties of the serotonin-sensitive receptors in organisms at various phylogenetic levels. It should be noted that in this case it is a matter of receptors with different physiological significance. The functional response to serotonin is probably based on the same reaction, independent of whether this substance is a local hormone with rhythmic activity (motor cells of mollusk embryos), a physiologically active factor carried by the blood (blood vessels of the rabbit ear), or a mediator of the nervous system (snail heart [4]).

The optimum conditions for the interaction of indolylamines with the receptors under consideration are the presence of an unchanged indole ring, a side chain in the 3-position, with a length of no more than 2 carbon atoms, and a substituent in the 5-position (the most active substituent is the hydroxy group). The reduction of the sensitivity of the receptors when substituents are introduced into other positions of the indole ring and into the side chain is probably due to a disturbance of the steric relationship of the receptor and the amine molecule. The increase in sensitivity when a substituent is present in the 5-position is evidently related to facilitation of the orientation of the amine molecule on the surface of the receptor.

SUMMARY

All of the studied indolylalkylamines (30 substances in all) as well as β-indazolylethylamine are capable of stimulating the embryonal motor activity of nudibranchiate mollusks. The most active in this respect is 5-hydroxytryptamine (serotonin). Combinations with substituting groups in the 2nd, 4th, 6th, or 7th position of the indol ring are less active than tryptamine. The activity is also lessened by elongation of the lateral chain (δ-3-indolylbutylamine), by inclusion of the methyl group in the α-position or by substituting nitrogen for carbon in the 2nd position of the indole ring. This reduction is the most evident in early mollusk embryos, when some indolylalkylamines are almost inactive. With the formation of innervation the sensitivity of the motor cells to serotonin and tryptamine declines and to a number of other indolylalkylamine increases. A similar series of activities has been discovered in the study of the vasoconstrictive effect of indolylalkylamine on the vessels of the rabbit ear.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
