ANNAL. BIOL. TIHANY	33	13—35	HUNGARIA 1966

CONTRIBUTIONS TO THE MECHANISM OF TRYPTAMINE EFFECT ON THE ADDUCTOR ACTIVITY OF FRESH-WATER MUSSEL LARVAE

E. LÁBOS

Biological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary

Received: March 3rd 1966

In previous investigations it has been demonstrated that tryptamine in a 10^{-6}—10^{-3} M/lit concentration induces a rhythmic activity of considerable degree on the adductor of fresh water mussel larvae (LÁBOS et al. 1964 a). Activity is either not induced at all by serotonine (5-HT) or it leads to an initial, rapidly subsiding activity. Tryptamine effect is changing, too. It is considerably lower in the case of late glochidia.

Tryptamine effect can be inhibited by SH-inhibitors, methionine, cysteine (LÁBOS et al. 1964 b).

In view of the fact that organ preparations of adult *Anodonta* are generally 5-HT, but not tryptamine sensitive (SALÁNKI 1963; FÄNGE 1955) and according to certain assumptions the various indolealkylamines penetrate differently through the mammal blood-brain-barrier (VANE et al. 1961), it seemed necessary to further examine the existing differences and changes in the effect of 5-HT and tryptamine. Experiments were directed in the first place to the action of agonists and antagonists that proved to be effective on cholinergic, adrenergic and other mediator systems. We also examined the effect of oxidase inhibitors to elucidate the metabolic processes essential in tryptamine effect. With this purpose in view we also demonstrate a photoreactive system in the presence of tryptamine, taking into consideration that the measure of some metabolic inhibitions may be light dependent even in not photosynthesizing systems. We consider in the case of embryonal object the comparative pharmacological examination of morphogenesis and embryonal motorics as a source of useful relationships and therefore conducted the examination of tryptamine effect also in an earlier ontogenetic stage.

Method

We gained the glochidia and earlier embryos from the external gill plates of adult animals, then selected them with the aid of a fine pipette into groups of 25 and within these groups examined the activity of the various animals for at most 60' in general. With each substance — in the way described earlier (LÁBOS et al. 1964 a) — we counted the contractions per minute and the larvae in tone at least in four groups. So the activity values are to be understood

on every *Figure* and concerning each material and material mixture for at least 100 glochidia each.

The materials used in the experiments were the following: tryptamine HCl (TA; Fluka), serotonin creatinine sulhpate (5-HT; Fluka), serotonin hydrogen oxalate (Calbiochem), Indole-3-yl-propionic acid (IPA; BDH), indole-3-yl-butyric acid (IBA; Schuchardt), indole-3-yl-acetic acid (IAA; Schuchardt), melatonine, bufotenine, 5-methoxy-tryptamine, L-andrenaline, L-noradrenaline, 3,4-dihydroxy-phenylalanine (DOPA), α-methyl-DOPA, dopamine, DL-iso-propyl-noradrenaline (IPNA), dichlor-isoproterenol (DCI), dibenamine HCl, acetylcholine chloride (ACh; Sandoz), mecholyl, carbamylcholine chloride (CaCh; Fluka), acetylthiocholine iodide (Fluka), butirylthiocholine iodide (Fluka), eserine-salicylate, neostigminium bromide (Merck), Mytolon (Win-2747), picrotoxin (Fluka) γ- amino butyric acid (GABA; Reanal), histamine, cystamine HCl, tyramiten HCl (Fluka), L-glutamine (BDH) KCl, $CaCl_2$, KCN, NaN_3 (BDH), 2,4-dintrophenol (2,4-DNP), iproniazid, isonicotinic acid hydrazide (INH), ergotamine tartarate, riboflavin-5-phosphate Na (FMN; Schuchardt).

Results

1. Some characteristics of the tryptamine effect.

The group reaction of glochidia shows in the presence of effective tryptamine concentration a frequency-time diagram taking place through maximum (LÁBOS et al. 1964 a). The rhythmic contraction series produced by the individual shows the same distribution in time *(Fig. 1)*. Thus the maximum observed in the group-reaction is not only the consequence of the averaging but also of the individual reaction. Therefore the formal modeling of the rhythm taking place through the maximum may supply information on certain questions. Let us consider the adductor as a system with in- and output. In the input there is an analogous process of the binding and chemical transformation of the substance employed (tryptamine), while on the output of the system there is a rhythmic contraction series. The rhythm appears after a certain period of latency, the frequency increases, for a time the contractions follow each other in regular intervals, subsequently the frequency diminishes and the glochidia generally remain open in a relaxed condition of their adductor. If the velocity of the input process is the frequency maintaining factor then its realization in time takes place along an S-shaped diagram the median linear sector of which corresponds to the rhythm of often remarkably stable frequency. Beside the rhythm pattern indicated in *Fig. 1* we also supplied the diagram of the assumed analogous process gained with graphical demodulation.

The maximum of the tryptamine dosage-effect diagram *(Fig. 2)* is in tryptamine sensible populations around a concentration of 100 μg/ml (type A). The reduction of sensitivity in spring described previously (LÁBOS et al. 1964) may be very considerable and is presumably connected with maturity because it can be observed also on late winter glochidia and those before getting out. In the autumn the maximum frequency is generally 10/min and 100 μg/ml tryptamine elicits 2000—4000 contractions out of 100 glochidia while in the case of insensible populations 500 μg/ml tryptamine causes 400—700 contractions and the 2—3/min individual maximum frequency also appears later

(type B). The data refer to examinations conducted at room temperature with an illumination of some thousand lx.

The further experiments were conducted on populations giving tryptamine response of partly A, partly B type. The concentration employed was

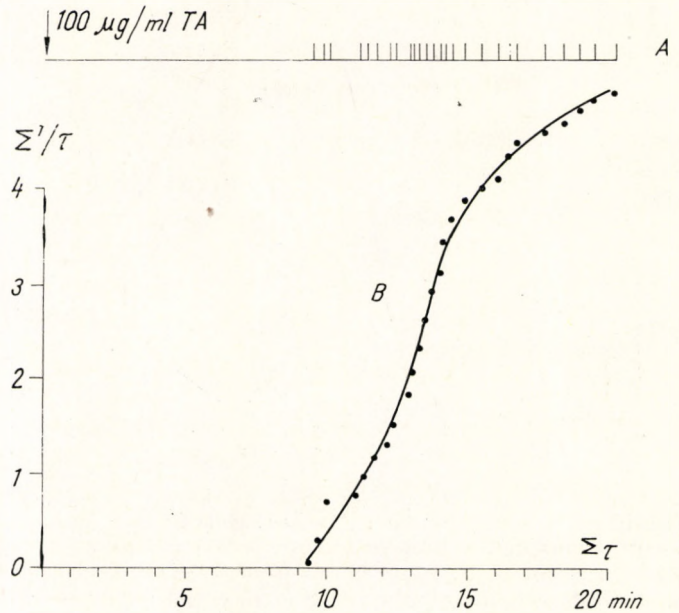


Fig. 1. Graphic demodulation of a rhythmic contraction series (upper line) induced by 100 µg/ml tryptamine on a single glochidium. *Abscissa:* time from the administration of tryptamine in minutes. *Ordinate:* sum of the $1/\tau$ values proportionate to the momentary frequency in arbitrary units, where τ_i is the time between the (i—1)th and i-eth contractions, observed after the administration. For further explanation see the text.

1. ábra Egyetlen glochidiumon 100 µg/ml triptaminnal kiváltott ritmikus kontrakciósorozat (felső vonal) grafikus demodulálása. *Abszcissza:* A triptamin adásától eltelt idő percekben. *Ordináta:* önkényes egységekben a pillanatnyi frekvenciával arányos $1/\tau$ értékek összege, ahol τ_i az adás után észlelt (i—1)-ik és i-edik kontrakció között eltelt idő. További magyarázatot lásd a szövegben.

50—100 or 500 µg/ml respectively and the value of the response pointed to the character of the population. With regard to the few months of difference between the appearance of the two populations not all examinations were carried out on both populations. Therefore in the course of the following the observation of pharmacological differences beyond the reduction of sensitivity may be expected.

2. The effect of indole derivatives

The effects of tryptamine, serotonin, melatonine, bufotenine, 5-methoxytryptamine, 3-indole acetic acid, 3-indole propionic acid, 3-indole-butyric acid were compared in 50—200 µg/ml concentrations.

Of the substances examined tryptamine and 5-methoxytryptamine are effective *(Fig. 3)*.

Serotonin and the other compounds elicit insignificant activity. Both preparations of serotonin used (creatinine sulphate and oxalate) are inefficient. In some cases and in higher concentrations serotonin induces higher initial activity which gradually subsides while in other cases this phenomenon also

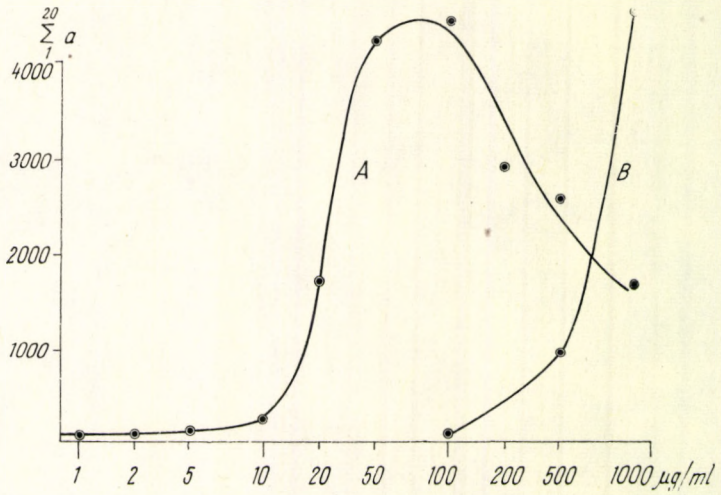


Fig. 2. Diagram of the tryptamine dosage effect in the case of populations sensitive (A) and less sensitive (B) to tryptamine. *Abscissa:* time in min., *ordinate:* the number of rhythmic contractions in the first 20 minutes following the administration. Every point signifies the number of the actions of 100 larvae each.

2. ábra A triptamin dózis-hatás görbéje triptaminra érzékeny (A) és érzéketlenebb (B) populáció esetén. *Abszcissza:* idő min-ban, *ordináta:* az adást követő első 20 perc ritmikus kontrakcióinak száma. Minden pont 100—100 lárva akcióinak számát jelenti.

f

ails to come about. Even this minimum activity observed can be reduced if serotonin is administered together with flavinadenosine monophosphate (FMN) which forms a complex in the solution. The external interaction arising in the mixture diminishes the initial 5-HT rhythm which contradicts the assumption that the exchange of solution should be the cause of the activity. Tryptamine activity can be reduced too in the presence of FMN.

The effect of non-activating bufotenine and serotonin and 3-indole acetic acid was examined on 100 glochidia each upon tryptamine action. Bufotenine hardly or not at all while serotonin to a slight degree delays the tryptamine effect. The phenomenon may be based on the hardly explicit competition of the two substances — the ineffective 5-HT and the efficient tryptamine — in a penetration to the site of the action.

3-indole acetic acid given together with tryptamine potentiates its rhythm — inducing effect *(Fig. 4)*. At the same time the tone diagram observed in the presence of the mixture runs on lower values.

3. The effect of sympathicotropic pharmacons

In our previous examinations we demonstrated that adrenaline, noradrenaline, tyramine do not induce rhythmic and tonic adductor activity (LÁBOS et al. 1964 a).

In the present investigations we examined in 50—100 μg/ml concentration the action of DOPA, α-methyl-DOPA, dopamine, iso-propyl-noradrenaline (IPNA), ergotamine, dibenamine, dichloro-iso-propyl-noradrenaline (DCI), adrenaline, noradrenaline on the adductor activity of glochidia and on the rhythmic response of the adductor caused by tryptamine.

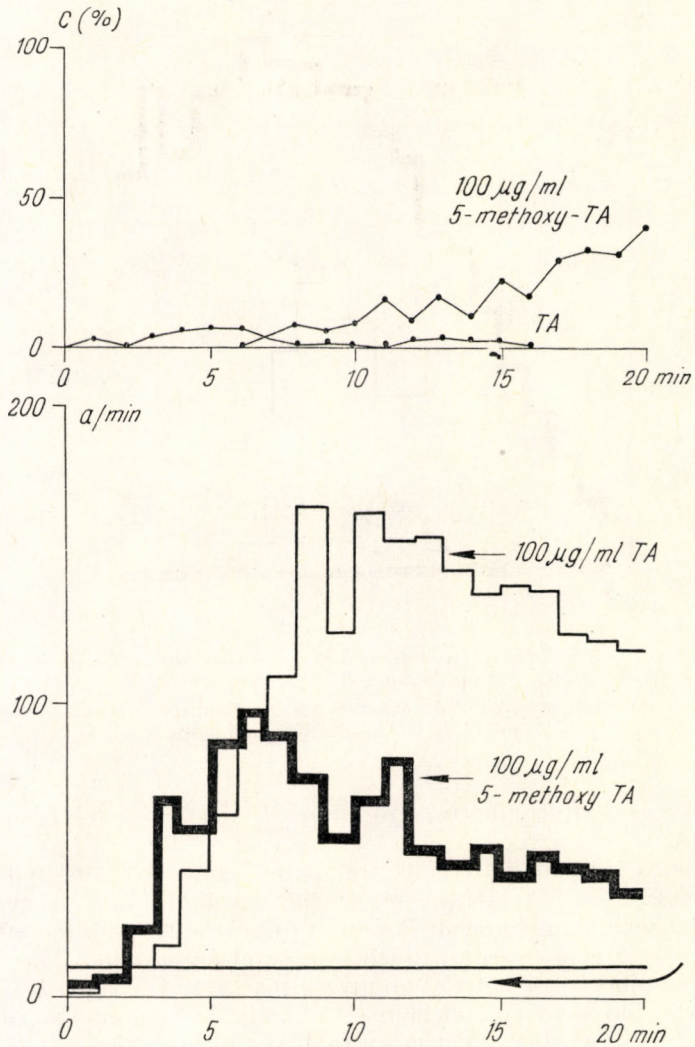


Fig. 3. Rhythmic activity and tonic closure inducing effect of 100 μg/ml tryptamine, 5-methoxy-tryptamine and 5-HT. *Abscissa:* time in min., *ordinate:* frequency of the rhythmic activity of 100 animals (a/min) and the proportion of the tonically closed larvae, c[%]. Population of intermediary sensitivity.

3. ábra 100 μg/ml triptamin, 5-methoxy-triptamin és 5-HT ritmikus aktivitást és tónusos zárást kiváltó hatása. *Abszcissza:* idő min-ban, *ordináta:* 100 állat ritmikus aktivitásának frekvenciája (a/min) illetve a tónusosan zárt lárvák aránya, c[%]. Átmeneti érzékenységű populáció.

In themselves, in concentrations of 10—100 μg/ml DOPA, α-methyl-DOPA, dopamine, IPNA on the basis of a 30 minute examination generally do not induce either rhytmic or tonic response on B-type population. Some

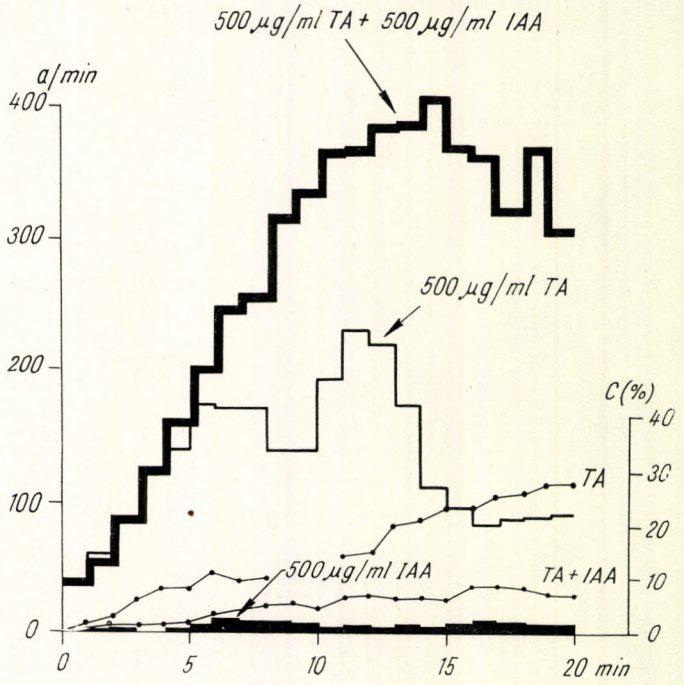


Fig. 4. Effect of 3-indole acetic acid (IAA). *Abscissa* and *ordinate* as in *Fig. 3.* Population of the B-type.

4. ábra Nagy koncentrációjú 3-indolecetsav (IAA) potencírozó hatása. *Abszcissa* és *ordináta*, mint a *3. ábránál.* B-típusú populáció.

populations gave a rhythmic response of low frequency in the presence of 100 μg/ml DOPA.

Noradrenaline, adrenaline in 100 μg/ml concentration potentiate the tryptamine response. The frequency of the rhythmic activity substantially increases, the tone is unchanged. Potentiation of the tryptamine effect can be also observed in the presence of dibenamine and ergotamine. The dibenamine effect was examined on a population giving reaction of A-type. Ergotamine in itself causes initial activity and increase of tone. Dibenamine in itself is ineffective, inducing neither rhytmic nor tonic response. Action of the α-receptor agonist and its antagonists is presented in *Figs 5* and *6.*

Examinations were conducted at room temperature with some thousand lx illumination in a neutral medium, thus, the decomposition of catecholamines accompanied by colouration took place in spite of the preparation of fresh solution.

The effect of IPNA differs from that of adrenaline and noradrenaline in that the potentiation appears later. The initial sector of tryptamine effect is

inhibited in the presence of IPNA, while the tryptamine effect extends to almost 1 hour. Potentiating dominates. The own effect of IPNA is insignificant, the tone unchanged. Results are presented in *Fig. 7.*

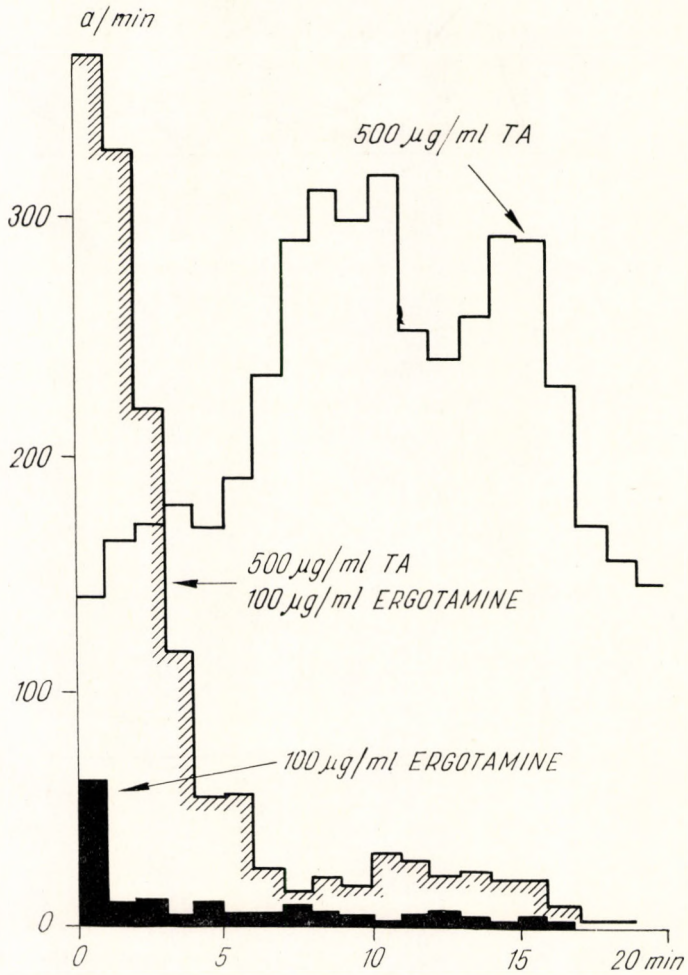


Fig. 5. Action of ergotamine on the tryptamine rhythm. *Abscissa* and *ordinate* as in *Fig. 3.*
5. ábra Ergotamin hatása a triptamin-ritmusra. *Abszcissza* és *ordináta* mint a *3. ábránál.*

DCI in itself depending on the cocentration is able to elicit a rhythmic activity of very high grade, without increasing the tone during that time. Thus 50 µg/ml DCI includes a rhythmic acitivity lasting for 10 minutes, with a peak in the 4—6th minute. This rhythmic activity almost completely subsides by the 10th minute. Until the 100—200th minute of the examination, however, almost 100 per cent of the glochidia get gradually into tonic contraction *(Fig. 8).* DCI increases the activity already in a concentration of 10 µg/ml ($\sim$ 40

μ M). This activity reaches its maximum in the 1—4th hour of the examination. No increase of tone occurs. In 100—200 μg/ml DCI the increase of activity takes place during the first 3—5 minutes of the examination. The time needed

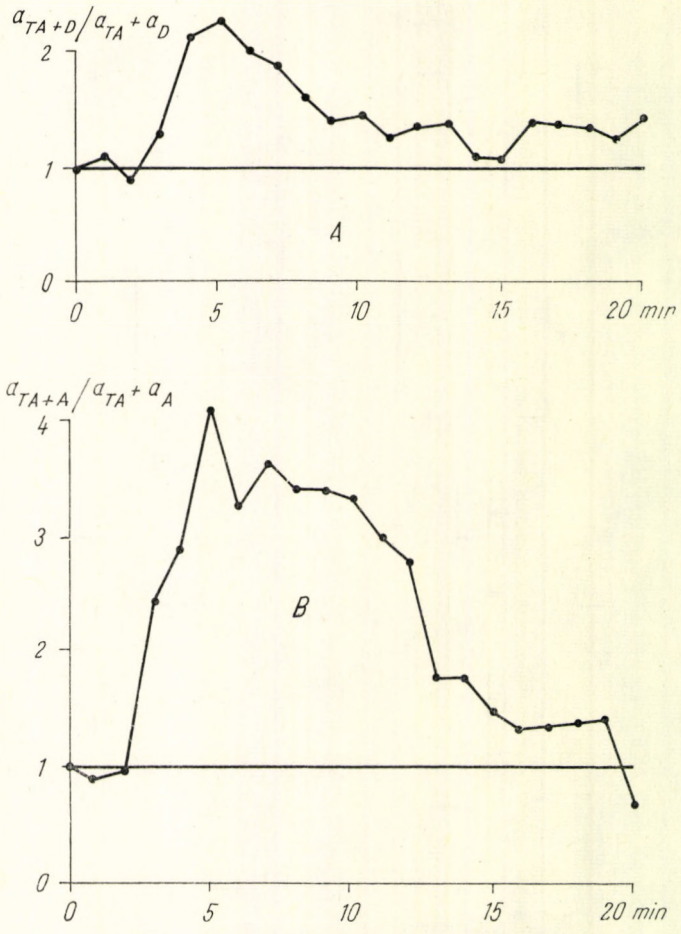


Fig. 6. A) Proportion of frequencies (a_{TA},a_D,a_{TA+D}) observed at the combined and separate administration of 50 μg/ml tryptamine and 70 μg/ml dibenamine (D)
B) Proportion of frequencies (a_{TA},a_A,a_{TA+A}) observed at the separate and combined administration of 500 μg/ml tryptamine and 100 μg/ml adrenaline (A)

6. ábra A) 50 μg/ml triptamine és 70 μg/ml dibenamine (D) együttes és külön történt adásakor észlelt frekvenciák aránya (a_{TA},a_D,a_{TA+D}).
B) 500 μg/ml triptamin és 100 μg/ml adrenaline (A) külön és együttes adásakor észlelt frekvenciák aránya (a_{TA}, a_A, a_{TA+A}).

for the closing of 50 per cent of the glochidia is 10—35 minutes. In the non-buffered system the pH was practically neutral. Thus the action of DCI may be considered as specific. When given together with tryptamine (100 μg/ml TA + 100 μg/ml DCI) a frequency exceeding the sum of the effect of the two substances measured separately can be observed.

Rhythmic activity caused by tryptamine is not substantially influenced by α-methyl-DOPA while it is somewhat inhibited by dopamine. Triptamine was employed in 500 μg/ml, α-methyl-DOPA and dopamine in 100 μg/ml concentration.

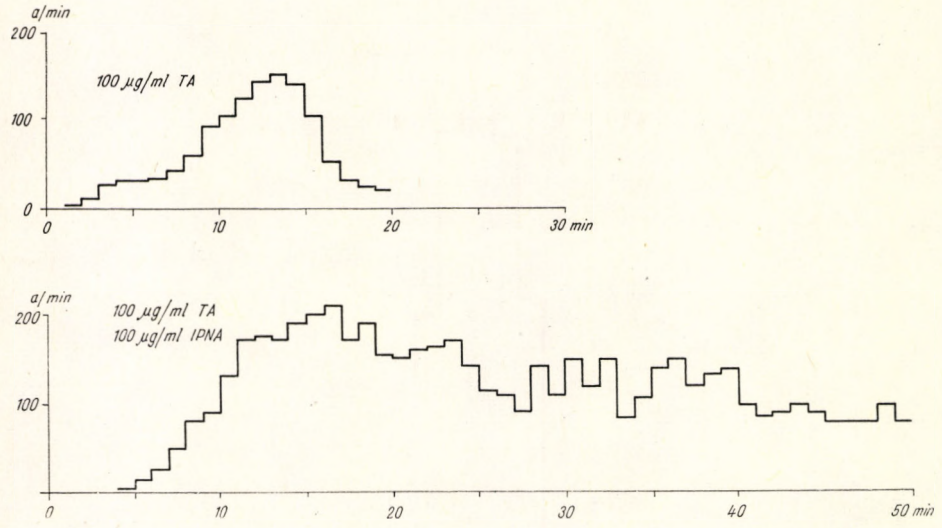


Fig. 7. Effect of isopropyl-noradrenaline (IPNA) on the tryptamine rhythm. For *abscissa* and *ordinate* see *Fig. 3.*

7. ábra Izopropil-noradrenaline (IPNA) hatása a triptaminritmusra. *Abszcisszát* és *ordinátát* lásd a *3. ábránál.*

4. The effect of cholinesters, cholinesterase-inhibitors, mytolon, picrotoxin, GABA, glutamine, histamine, cystamine, KCl, $CaCl_2$ on the rhythmic activity and the tryptamine rhythm.

Various cholinesters — acetylcholine, carbamylcholine, acetyl-β-methyl-choline, acetyl- and butyril-thiocholine, benzoylcholine in themselves induce neither lasting rhythmic activity nor tone. ACh in some cases elicits a rapidly (in 1—3 min) subsiding rhythm in high — 10^{-4} — 10^{-3} M/lit — concentrations. At room temperature and pH—7—8, with 4—5 mM substrate concentration in the complete homogenizate of 200 mg glochidium there is no measurable ACh-hydrolysis in 1—3 hours, and at 37°C only a few, which does not explain the ineffectiveness of ACh. When givin ACh in 1 m M concentration together with tryptamine, the tryptamine-rhythm arises to an unchanged degree *(Fig. 9).*

Prostigmine, mytolon by themselves are ineffective. Eserine in 1 mg/ml concentration induces considerable rhythmic activity. Given together with tryptamine, their effect generally gets summarized. The effect of UV-irradiated eserine changes depending on the dosage rate of irradiation. The growth, decrease then new increase of the effect may be in connection with the action of various photolytic and hydrolytic intermediates *(Fig. 10).* The conditions of

the irradiation are: 250 W, Hg-lamp, 50 cm, in a quartz test tube, for a period of lo" — 60'. The new increase refers only to the frequency of the rhythm.

Mytolon given together with tryptamine prolongs its effect. Histamine, cystamine do not induce rhytmic activity. Cystamine potentiates the rhythm induced by tryptamine.

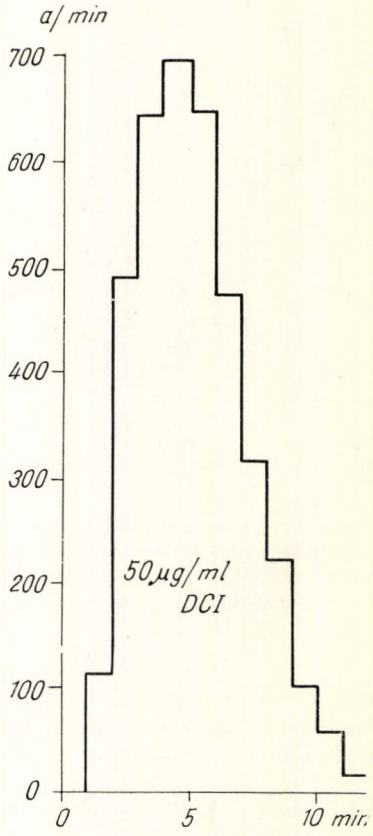


Fig. 8. Effect dichlor-iso-proterenol (DCI) For *abscissa* and *ordinate* see *Fig. 3.*
8. ábra Diklór-izo-proterenol (DCI) hatása. *Abszcissza* és *ordináta* mint a *3. ábrán.*

Picrotoxin in concentrations not higher than 100 µg/ml potentiates *(Fig. 11)* the tryptamine effect. γ-amino-butyric acid (GABA) and glutamine in 100 µg/ml concentration are ineffective by themselves and neither inhibit nor potentiate the tryptamine effect.

The KCl-tone increases with the presence of tryptamine. The rhythmic activity, when KCl and tryptamine are given togther, is indicated by the rhythm subsiding on account of the increase of tone; it lasts for a shorter time and becomes lesser as compared with the separate application of the two substances.

$CaCl_2$ in high (10—50 mM/lit) concentration blocks the rhythmic activity caused by tryptamine. The tone on account of the presence of $CaCl_2$ increases.

5. The effect on tryptamine response of agents acting on oxidative metabolic processes.

In our previous investigations we demonstrated the blocking effect of iproniazid in a short-term experiment on tryptamine-sensitive population (LÁBOS et al. 1964 a). In the course of the repetition of these experiments the

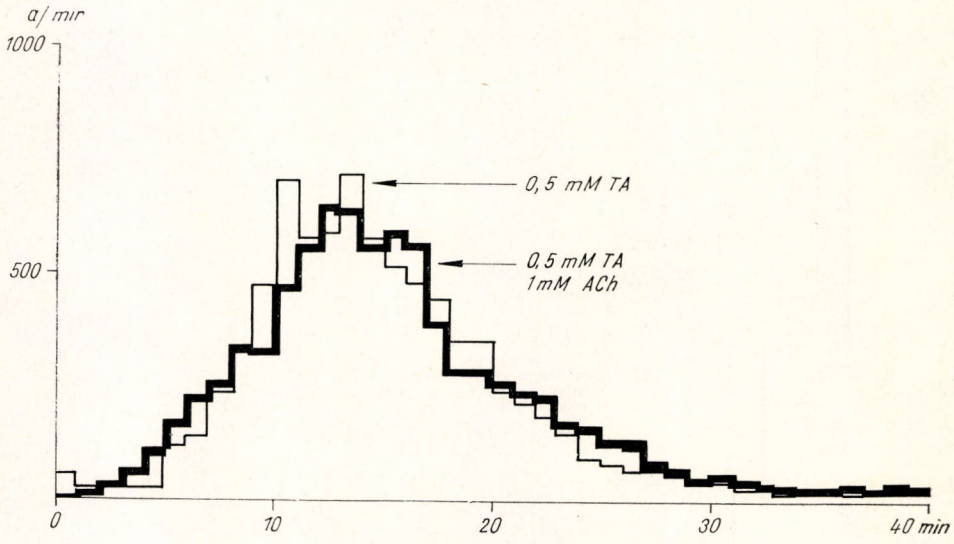


Fig. 9. Effect of acetylcholine (ACh) on the tryptamine rhythm (TA). For *abscissa* and *ordinate* see *Fig. 3.*

9. ábra Acetilkolin (ACh) hatása a triptamin-ritmusra (TA). *Abszcissza* és *ordináta* mint *a 3. ábrán.*

phenomenon proved to be essentially the delaying of the tryptamine effect. The equally monoamino-oxidase-inhibiting iso-nicotinic acid hydrazide (INH) exhibited still less blocking effect than iproniazide. In the 50—100 μg/ml concentration in a 20 minute experiment no potentiating was observed.

Conducting the examinations only on populations of the B type, 100 μg/ml $NaN_3$and 50 μg/ml KCN influence the tryptamine effect. In the case of KCN owing to its own effect the potentiating relates to the 1st and 2nd minute and manifests itself in an increase of about three times of the maximum frequency. In the case of NaN_3 potentiating of the maximum frequency is 8—10 fold*) Fig. 12)*. The own tone increasing effect of KCN dominates the tone-diagram which in the presence of tryptamine somewhat decreases. In the presence of NaN_3 + tryptamine mixture also the tone is potentiated.

From *Fig. 13* it appears that the otherwise slight effect of 5-HT is also potentiated by NaN_3. Essentially this is the appearance of a new effect, because 5-HT in itself is almost ineffective. In the presence of tyramine no potentiating comes into being.

2,4-DNP inhibits the tryptamine effect *(Fig. 14).*

6. Light sensitive system in the presence of tryptamine

The examinations outlined above were conducted in the presence of a 15 W microscopic tungsten lamp (50—70°, 120—140 mm). Measured with a mercury thermometer a heating up by 5° C/ hour was then observed. With the

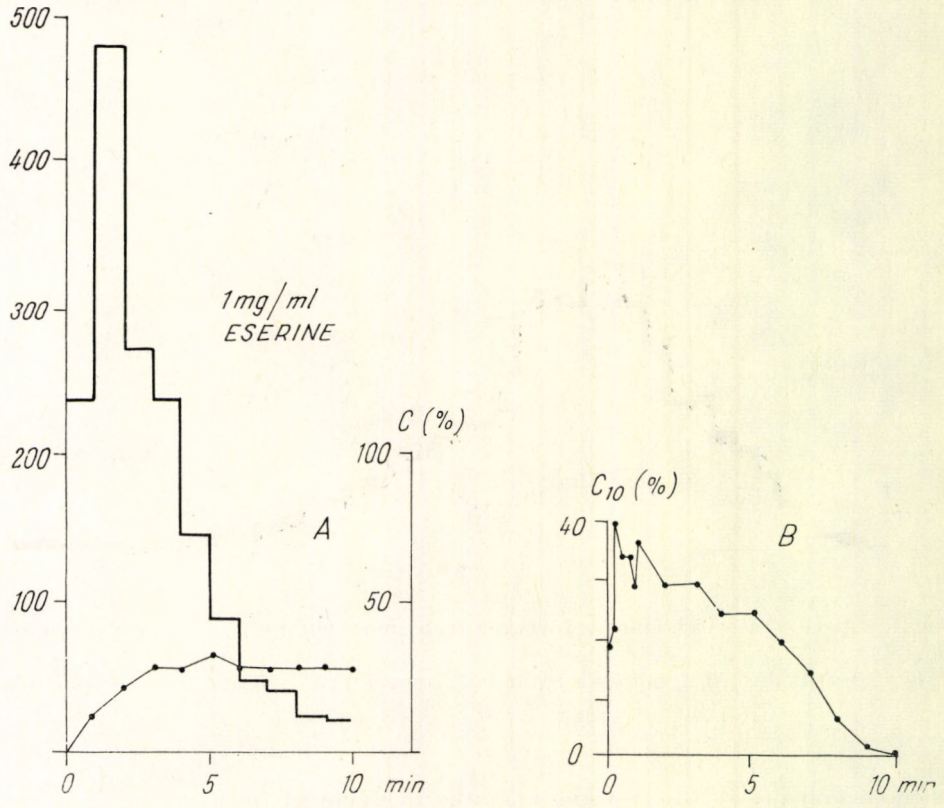


Fig. 10. A) Effect of eserine applied in high concentration. For signs see *Fig. 3.* B) Proportion of larvae found in tone in the 10th minute after the administration of irradiated 1 mg/ml eserine. On the *abscissa* the period of UV-irradiation.

10. ábra A) Nagy koncentrációban alkalmazott eserin hatása. Jelöléseket lásd a *3. ábrán.* B) 1 mg/ml eserin adása után a 10. percben tónusban talált lárvák aránya. *Abszcisszán* az UV- besugárzás ideje.

use of BG-17 infra filter this value was 3—4 C° hour. Thus the nominal temperature of the incubation mixtures rose by 3—5°C at the most.

We observed the following phenomenon: in the presence of 0.5 mM tryptamine, 1 mM monoiodide acetic acid, 10 mM methionine- after 20—50 minutes of incubation in this solution the rhythmic activity showed a very great difference depending on the illumination *(Fig. 15).* The light is of activating effect. The solution is not coloured as observed with the naked eye. The reaction takes place also in the presence of BG-17 infra filter and with ventilator cooling (evaporation). Without previous incubation the difference is less.

Of the three components only tryptamine has an activity increasing effect. Methionine and monoiodo acetic acid are, irrespective of the illumination, ineffective. The tryptamine effect on the other hand increases when the microscope lamp is lighted. This increase is lesser than in the case of the mixture, after a proper period of preliminary incubation.

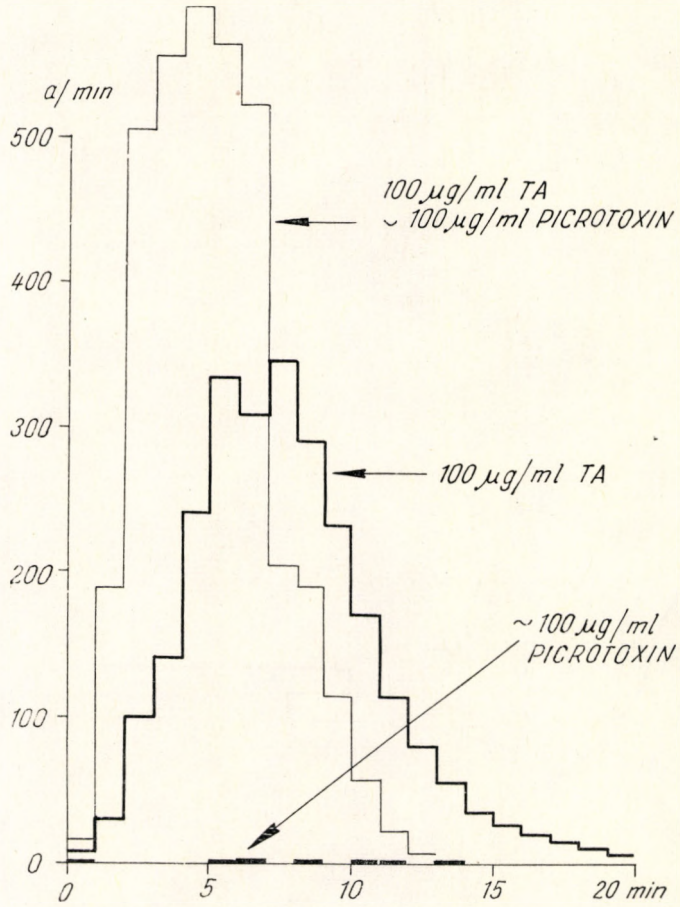


Fig. 11. Effect of 100 µg/ml picrotoxin on the rhythmic activity induced by 100 µg/ml tryptamine. Population of the A-type. For signs see *Fig. 3.*

11. ábra 100 µg/ml picrotoxin hatása a 100 µg/ml triptamin által kiváltott ritmikus aktivitásra. A-típusú populáció. Jelöléseket lásd a *3. ábrán.*

7. The effect of tryptamine on rotating forms

The glochidium stage is preceded by the so-called rotating embryonal stage when within membrane the embryo is rotating. For the rotation a ciliary zone is responsible on the surface of the embryo. The limits of the rotation are 20— 360. cph.

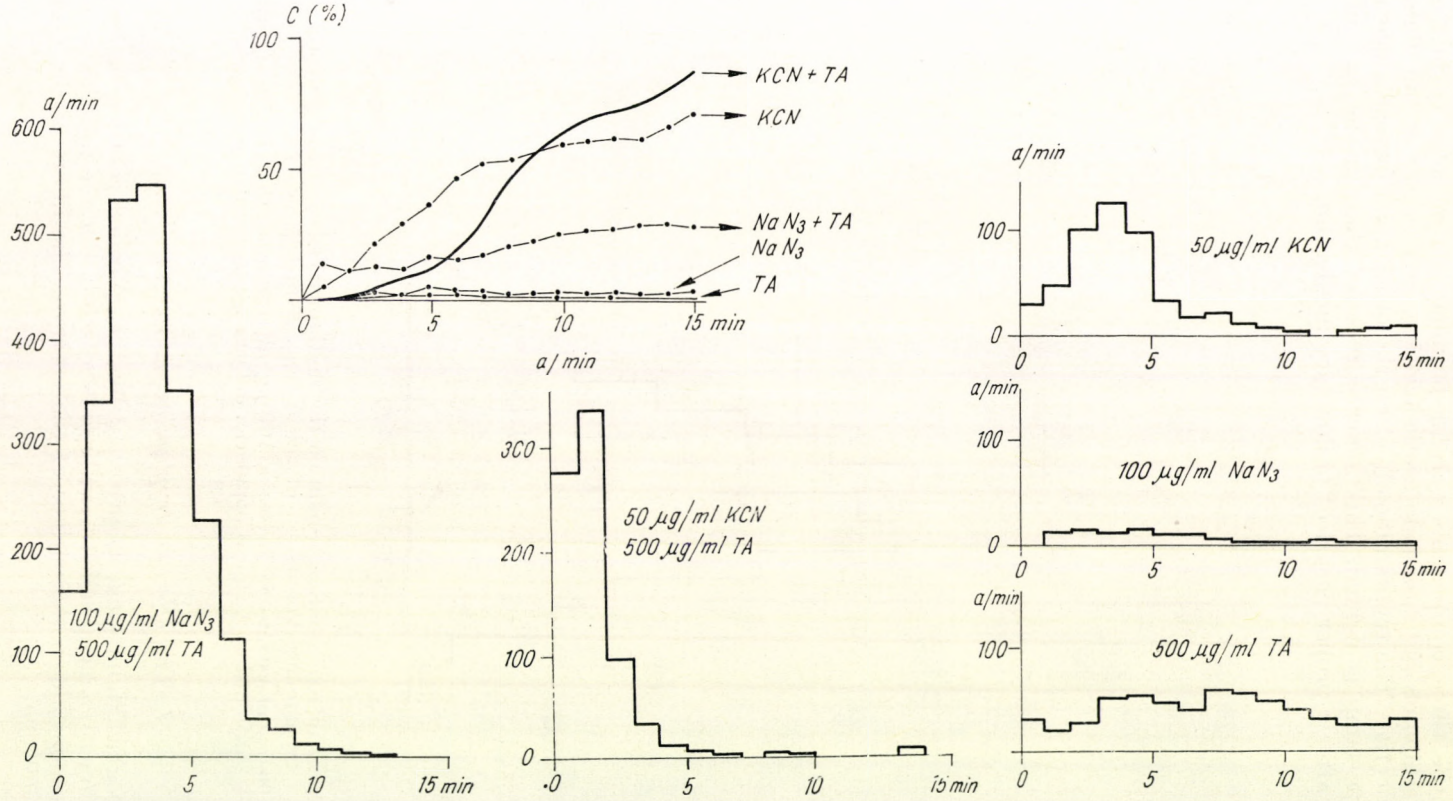


Fig. 12. Effect of KCN, NaN_3 on rhythm and tone induced by tryptamine. For sign see *Fig. 3.*

12. ábra KCN, NaN_3 hatása a triptamin által kiváltott ritmusra és tónusra. Jelöléseket lásd a *3. ábrán.*

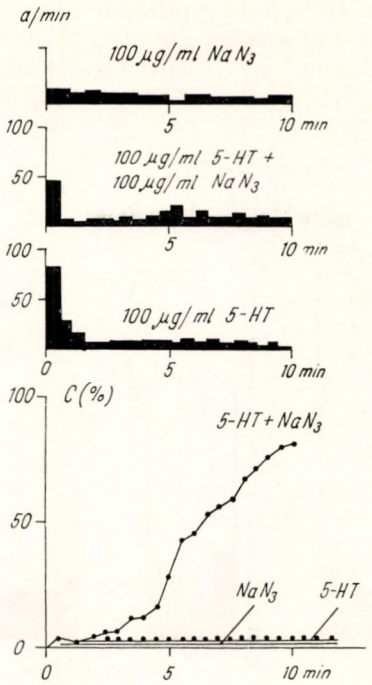


Fig. 13. Tonic closure at combined administration of 5-HT and NaN_3. For signs see *Fig. 3.*
13. ábra Tónusos zárás 5-HT és NaN_3 együttes adásakor. Jelöléseket lásd a *3. ábrán.*

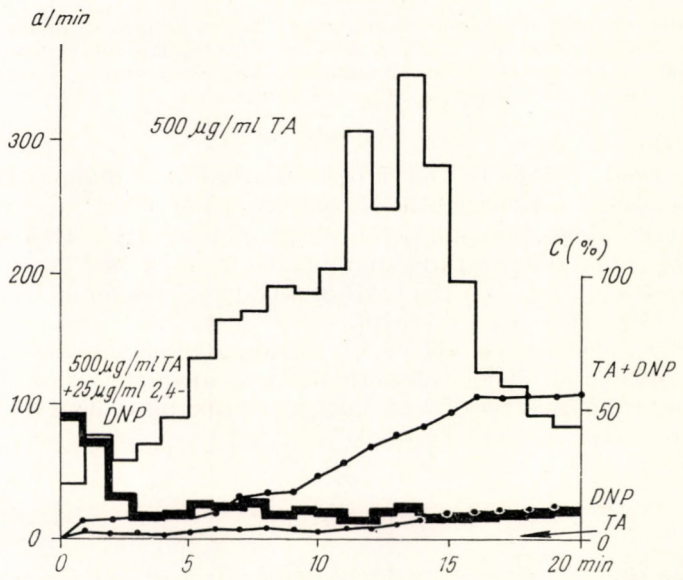


Fig. 14. The inhibition of 500 µg/ml tryptamine with 25 µg/ml 2,4DNP.
For signs see *Fig. 3.*
14. ábra 500 µg/ml triptamin hatásának gátlása 25 µg/ml 2,4-DNP-lal. Jelöléseket lásd a *3. ábrán.*

In the presence of 10 μg/ml tryptamine after a transitory acceleration (which in view of its instantaneous appearance is regarded as non specific) therotation after a few hours comes to a stillstand and the following phenom-

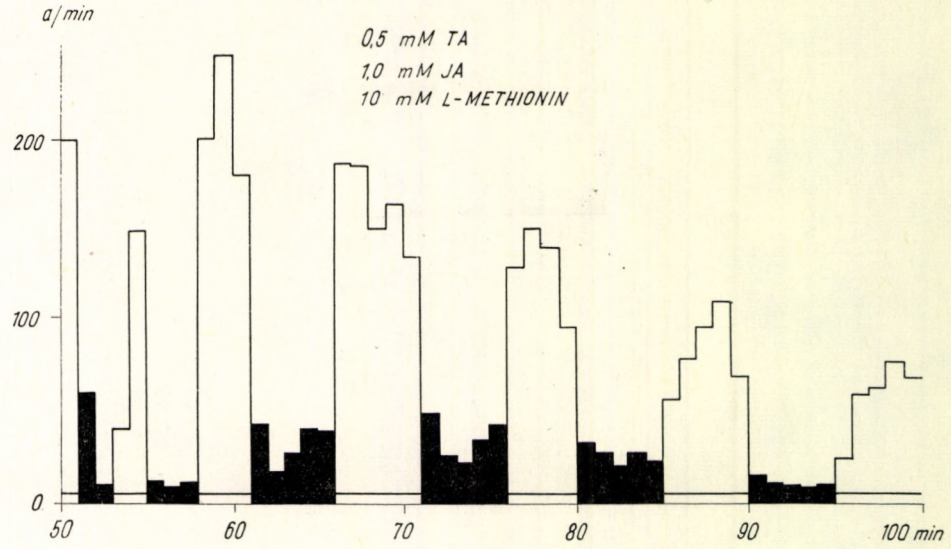


Fig. 15. Frequency of the rhythmic activity of 25 glochidia with alternating light and darkness. For signs see *Fig. 3.* Dark areas refer to dark, light areas to light time periods. Composition of incubation mixture: tryptamine, monoiodo-acetic acid JA and L-methionine

15. ábra Váltakozó megvilágítás és sötét mellett 25 glochidium ritmikus aktivitásának frekvenciája. Jelöléseket lásd a *3. ábrán.* A sötét területek a sötét, a világos területek a világos időszakaszokra vonatkoznak. Inkubációs elegy összetétele: triptamin, monojód-ecetsav (JA) és L-metionin.

enon is observed: within the larva is surrounded by a marginally branching cell mass connected with each other loosely or not at all.

Of other indole compounds the effect of IAA, IPA, IBA and 5-HT is examined at room temperature, in room light, in 2 mM/lit concentration.

The action sequence on the basis of 5 hours of mesaurement:

TA > IPA, IBA, IAA > 5-HT.

The effect also sets on when a very dense embryo suspension is standing for a few hours in a physiological solution. In an adequately diluted suspension the embryos for hours invarably conduct a rotating movement and no signs of "dysmorphogenesis" are observed.

Discussion

A main characteristic of the tryptamine effect is that the termination of the rhythm induced generally takes place in a relaxed condition of the adductor. The group reaction shows a time diagram running through maximum frequency.

Considering that in spite of this the individual reaction can be manyfold, it was desirable to examine also the individual rhythm. The individual rhythm appears after a latency period, its frequency increases and after a comparatively stable interval it decreases. We discuss this phenomenon according to a widespread digitalization principle. The development of the rhythm is conceived as a counting, similar to a frequency modulation mechanism (NEUMANN 1948). For increase and decrease of the frequency the velocity of the process is responsible. Then the modulating factor must show an S-shaped time diagram, the median, nearly linear sector of which corresponds to the stable rhythm. This mechanism does not demand exact elementary oscillators, only a nearly linear physical or chemical process for the origin of the reactions sometimes following each other with remarkably exactitude to be explained. This linear sector is the median part of the process S. This process can be manifold, e. g. permeation, oxidation or other chemical transformation.

To test whether the oxidation of tryptamine through indole acetaldehyde into indole acetic acid may explain the stop of the rhythm induced by indole acetic acid production we employed tryptamine together with indole acetic acid and found that indole acetic acid far from inhibiting even potentiates the tryptamine effect. Thus, it is not indole acetic acid production but some other chemical transformation which is responsible for the cessation of the rhythm. This is supported by the finding according to which the indole acetic acid (heteroauxin) is of lesser "morphogenetic" effect than tryptamine. Consequently some other reaction of tryptamine must be responsible also for the effect of the latter.

Similar phenomenon is the decrease of tryptamine effect with the progress of ontogeny. In this case also the development of a mediator system not based on indoles can be assumed simultaneously with the development of the innervation of the adductor. The ineffectiveness of cholinesters and that of ACh also observed when administered together with tryptamine *(Fig. 8)* argues against the cholinergic character of the inhibiting system in question. This is supported also by the slight ChE-activity. Similarly the examinations of PHILLIS (1966) on *Tapes* heart do not point, to the possibility of a cholinergic system functioning not with ACh. The activity observed in the presence of high eserine concentration is attributed to the fact that eserine is an indole derivative and on the 5th C atom of the indole nucleus, there is a methyl carbamic acid ester bond. The comparison of 5-HT, tryptamine, 5-methoxy-tryptamine pointed to the effect of the two latter compounds. According to VANE and coworkers (1961) the latter two substances penetrate easier thorugh membranes. From present observations the great effect — modifiyng role of the substitution on the C atom No 5 is clear, but we do not see the reason, steric or originating from charge, of the inhibition of permeation. The reactivity of the group on the 5th C atom seems to be more important. AXELROD (1962) pointed to the importance of the O and N-methyl transferase systems of the biogenic amines. This, beside or instead of its permeation — regulating role may supply a point of support for the effectivity of the 5-methoxy-tryptamine as against the ineffectiveness of the 5-HT. The efficiency of tryptamine is not clear. SAKHAROV and PÉCSI (1965) on *Anodonta* heart observed the protective effect of 5-HT from heat inactivation. Tryptamine and 5-methoxy-tryptamine were of considerably lesser effect in the same phenomenon. The inverse sequence of action stressed instead of permeation the importance of other factors. In the

excitatory processes of Molluscs catechol- and indole-alkylamines are supposed to be widespread mediators. Among them, concerning effect and occurrence tryptamine is less outstanding than 5-HT (CARDOT and RIPPLINGER, 1963; DAHL et al. 1962). Our present examinations on the other hand stress the specificity of the tryptaminerg activating effect against the indole and phenyl-alkylamines examined.

Among the theoretically possible causes of the decrease of sensitivity also inhibition based on gamma-amino butyric acid system could be assumed. Picrotoxin, which is of convulsive effect on Crustacean peripheric and central preparations and in the same place specific blocker of inhibitions (HICHAR 1960) increases the activity of the glochidia caused by tryptamine. The gamma-amino butyric acid, however, does not inhibit the same. FLOREY and CHAPMAN (1961) pointed out that in Crustaceans the inhibiting factor is not unconditionally GABA. Our present result also seems to point out that behind the potentiation of picrotoxin no GABA-erg mechanism should be sought. The ineffectiveness of glutamine (as a blocking agent) excludes also the presence of the glutaminergic inhibiting system.

Adrenaline and noradrenaline are of potentiating effect. DOPA α-methyl-DOPA, dopamine are ineffective, respectively the latter is slightly inhibiting. The effect of IPNA can be also regarded as potentiation.

The antagonists of the α- and β-receptors, dibenamine, ergotamine and DCI potentiate or induce tonic or rhytmic activity respectively. The effect of antagonists of this type points to the fact that the tryptamine response of the A and B type can be increased by α- and β-adrenolytic interference. Especially-explicit is the own activating effect of DCI. Since, however, no definitely inhibiting sympathic agonist was found among those examined it may be assumed that a catecholamine not applied here may be responsible for the decrease of tryptamine effect in spring.

The potentiating effect of the various catecholamines raises the possibility of the release of activating catecholamine under the influence of tryptamine. BUNAG and WALASZEK (1963) have brought the two-phase character of the vascular effects of serotonin into connection with the release of two secondary mediators of antagonistic effect. It is an open question whether in our case a so much combined system may prevail. In this case the activating effect of tryptamine could be regarded as catecholamine mobilization. In this hypothesis no inhibiting catecholamine ought to be postulated. There are simple peptides e. g. glycyl-glycine which block the tryptamine effect in 1 mM concentration.

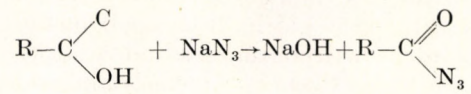
Finally it can not be left out of consideration in the evaluation of the potentianting effects of agonists that in the course of examination a number of coloured catecholamine oxidation products developed. BORG (1965) regards the development of the phenoxy- and indole derivative free radicals as an important primary step in the mode of action both of catechol and indole-alkylamines. Taking into consideration the reversibility of the one-electron oxidation, changes of action can arise in the course of oxidation as demonstrated in the photo-oxidation of phenothiazines (LÁBOS AND TURCSÁNYI 1965).

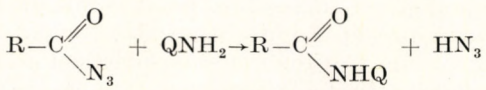
The effect of activating monoamines is potentiated by the inhibition of the oxidase ferments responsible for their decomposition. In mammal nervous system the inhibition of this widespread ferment induces excitation phenomena. Our previous finding on the inhibiting effect of the MAO-inhibiting

iproniazide (LÁBOS et al. 1964 a) and literary data (KARKI et al. 1962) on the ontogeny of the MAO-ferment (MAO level appearing and increasing with age (contradict the assumption that the energy released during tryptamine oxidation should be responsible for the increase of activity. This finding of ours on the other hand that cysteine inhibits the activity brought about by tryptamine (LÁBOS et al. 1964 b) argues for radical reactions and thus indirectly for the participation of oxidases.

The effect of KCN, NaN_3 and the activity inducing effect of KCN to the participation of an oxidase system. The effect of KCN substantially differs from that of KCl applied in the same concentration. The former leads to a tone and the rhythm simultaneously subsides (see *Fig. 12)* while the latter under a 1 mM/lit concentration does not lead to a tone. Thus the K- ion effects are not sufficient for the explanation of the phenomena accompanying the KCN action. The effect is a potentiating of a considerable degree in which the effective product argues for oxidative breakdown. More difficult to explain is the phenomenon that the NaN_3 potentiation can be observed also in the presence of the hardly effective 5-HT. The azide-potentiation can thus be interpreted also as block of relaxation. The hydrolysis of KCN also could not be neglected in the production of tonus.

The azide-potentiation is not specific for the tryptamine effect, so it can be assumed that the azide non specifically reacts with amines and the HN_3 developed in the reaction (STANNARD 1939) is responsible for the effects. The reaction is well known in organic chemistry (BRUCKNER 1965). The azide-acids in vitro react under comparatively mild conditions with amines since the N_3-group is very reactive and rich in energies.





In biochemistry for azide effects generally the development of HN_3 is made responsible for oxidase inhibitons (ref. HEWITT AND NICHOLAS 1963, STANNARD 1939). If the mechanism of the phenomenon is the outlined then binding of the amine in question must take place. The azide action led in the case of tryptamine, serotonin, to potentiating while in the case of tyramine this phenomenon failed to come about and in the case of serotonin even a tone, while in the case of tryptamine rhythm increase was observed. Therefore in our opinion the development of HN_3 in itself is no sufficient explanation and its bond with the amine — CO — NH— group might be a passible factor, too. This is of different effect in the case of the various amines.

The effect of 2,4-DNP stresses the significance of oxidative phosphorylation. The above azide-reaction also takes place more readily in the presence of activated i. e. esterified R—COOH group.

At present no other explanation is available for azido-potentiating which contains also specific for the various amines.

The photo-activation observed in the presence of methionine and monoiodo ethanoic acid can not be explained at present but it is evident that it takes

place on a coloured system. Since it is also supplied by tryptamine alone to a lesser degree, therefore the tryptamine binding can be already in connection with coloured substances. The KCN effect pointing to the participation of the cytochrome system and the coloured products which can be easily developed from tryptamine stress the possbility of the realization of a photochemical system.

BORG (1965) assumes free radicals in the first phase of the effect of biogenic amines. Our results on account of the realizability of the photoactivable system support the free radical formation. For this argues our preliminary finding according to which cysteine already in a concentration of 1—2 mM/lit readily inhibits the activating effect of tryptamine (LÁBOS et al. 1964 b). The potentiating and inhibiting effects of azide, cyanide active on oxidase systems and metalloferments and of 2,4-DNP decoupling oxidation from phosphorylation also argue for radical reactions. Consequently they support those hypotheses which assume behind the term "receptor" in the pharmacological sense a combined metabolic chain in which the electron-transfer reactions and phospholipids are deeply involved (DIKSTEIN and SULMAN 1965 a. 1965 b; WOOLEY and GOMMI 1964).

In view of the fact that in the ontogeny of glochidia the metamorphosis is preceded by a change in motorics namely lasting tone, the comparative pharmacological analysis of morphogenesis and embryo-motorics seems to be justified. BUZNYKOV (1963—64) developed incubation mixtures containing indole-compounds influencing both the motorics and morphogenesis of larvae. On the basis of his results and assumption the humoral mediators of ontogeny may include in an undifferentiated form also the specializing nervous transmitter systems. Further the fact that the oxidative desamination of tryptamine rather specifically activating the glochidium mechanics leads to the development of one of the plant growth hormones indole acetic acid (hetero-auxin) points to some common traits of morphogenesis and motorics. The development of amine incorporation and peptide bond assumed in the potentiating effect of NaN_3 motorics directs the attention also toward morhogenesis. Our unpublished data according to which chloramphenicol and aureomycin elicit motoric activity which are definitely substances influencing protein and nucleic acid synthesis, similarly require the examination of the common mechanism of embryonal motorics and morphogenesis.

According to our experiments the embryonal development preceding the glochidium stage can be disturbed with certain indole-compounds. Tryptamine also here proved to be more active than other derivatives.

Summary

The rhythmic motoric activity induced by tryptamine of the glochidia of the fresh water mussel *(Anodonta cygnea L.)* was examined alone and in the presence of various pharmacons. For the modeling of the rhythm a counting off mechanism was assumed. The cause of the subsiding of the rhythm could not be the development of indole acetic acid, because this agent potentiates the tryptamine effect.

By the examination of various indole derivatives we stress te specificity of the tryptamine effect. In concentrations of 10^{-4}—10^{-3} M/lit 5-methoxy —

tryptamine and eserine are indole derivatives of minor action but effective. Indole alkylic acids, melatonine, bufotenine are ineffective.

To explain the reduction of sensitivity observed with the progress of ontogey we assumed the development of a new mediator system. This can not be a cholinergic, GABA-ergic, glutaminergic system, since ACh,GABA, glutamine do not inhibit the activity caused by tryptamine. The application of sympathicolythica, however, argues for the development of a catecholamine inhibiting system. Dibenamine and ergotamine potentiate the tryptamine effect. Dichlor-isoproternol in itself is of activating and given together with tryptamine of potentiating effect. Of the catecholamines examined adrenaline, noradrenaline, IPNA are potentiating, while at tryptamine insensitive population dopamine, DOPA, α-methyl-DOPA are ineffective. Thus, the inhibiting catecholamine which can be made responsible for the activating effect of the sympatholytics is not among these.

It can be assumed that in the course of ontogeny also inhibiting and activating systems based on adrenergic mediation develop.

The effects of NaN_3 KCN and the inhibiting action of 2,4-DNP stress the importance of the oxidative metabolic processes. The mechanism of the azide action was discussed. The cyanide effect suggests the direct role of terminal oxidation. Photo-activation developed in a special incubation mixture (tryptamine + methionine + mono-iodo-acetic acid) also assumes the participation of a coloured (cytochromic?) system and stresses the importance of mechamisms with free radicals.

Action with indole-compounds can be demonstrated also on the morphogenesis of earlier embryonal forms. Tryptamine is also here of outstanding effect and in 100 μg/ml concentration brings about dysmorphogenesis, swel_ing and desintegration.

REFERENCES

Axelrod, J. (1962): The enzymatic N-methylation of serotonin and other amines. — *J. Pharmacol. Exp. Ther.* **138,** 28–33.

Borg, D. C. (1965): Transient free radical forms of hormones: EPR Spectra from Catecholamines and Adrenochrome. EPR Spectra from Iodo-thyronines, Indoles, Estrogens and Insulin. — *Proc. Nat. Acad. Sci.* **53,** 633–639 and 829–890.

Bruckner, Gy.: Szerves kémia I/1. — Organic chemistry I/1). *Tankönyvkiadó, Budapest* 1961.

Bunag, O., Walaszek E. J. (1963): The latent vasopressor effect of serotonin in the chicken. — *Arch. int. Pharmacodyn.* **143,** 108–117.

Buznyikov, G. A.: (personal communication).

Cardot, J., J. Ripplinger (1963): Mise en évidence de la triptamine dans les extraits nerveux du Mollusque *Helix pomatia.* — *Compt rendus. Soc. Biol.* **107,** 853–854.

Dahl, E., B. Falck, M. Lindquist, C. Meklenburg (1962): Monoamines in mollusc neurons. — *Kungl. Fysiografiska Sällkaptes i Lund Förhandlingar* **32,** 89–92.

Dikstein, S., F. G. Sulman (1965): Function of Phospholipids in Receptors. — *Biochem. Pharmacol.* **14,** 739–742.

Dikstein, S., F. G. Sulman (1965): Interaction of Dibenamine with phospholipids of the aorta. — *Biochem. Pharmacol.* **14,** 881–885.

Fänge, R. (1955): Use of the isolated heart of a fresh-water mussel *(Anodonta cygnea L.)* for biological estimation of 5-hydroxytryptamine. — *Experientia* **11,** 156.

Florey, E., D. D. Chapman (1961): The non-identity of the transmitter substance of crustacean inhibitory neurones and gamma-amino butyric acid. — *Comp. Biochem. Physiol.* **3,** 92–98.

HEWITT, E. J., D. J. D. NICHOLAS: Cations and Anions. Inhibitions and Interactions in Metabolism and in Enzyme Activity. — in: HOCHSTER, R. M., J. H. QUASTEL (1963): *Metabolic Inhibitors* **2,** Chapter 29.

HICHAR, J. K. (1960): Effects of γ-amino butyric acid and picrotoxin on spontaneous activity in the central nervous system of the crayfish. — *Nature, London.* **188,** 1117—1119.

KARKI, N., R. KUNTZMAN, P. B. BRODIE (1962): Storage, synthesis and metabolism of monoamines in the developing brain. *J. Neurochem.* **9,** 53—58.

LÁBOS, E., J. SALÁNKI, K. S. RÓZSA (1964a): Effect of serotonin and other bioactive agents on the rhythmic activity in the glochidia of fresh-water mussel *(Anodonta cygnea L.).* — *Comp. Biochem. Physiol.* **11,** 161—172.

LÁBOS, E., K. S.—RÓZSA, J. SALNÁKI (1964b): On the role of SH-groups in the rhythmic activity of adductor muscle of glochidia. — *Annal. Biol. Tihany* **31,** 39—54.

LÁBOS, E., B. TURCSÁNYI (1965): Photoinduced muscle-response of embryonic motor-system sensitized by xanthene-dyes. *Lecture in the 2nd Meeting of Hungarian Biochemical Society. Budapest* 1965. May 27th—29th.

NEUMANN, J. (1948): The general and logical theory of automats. *Lecture at the Hixon symposium (Pasadena, California,* 1948. IX. 20.)

PHILLIS, J. W. (1966): Innervation and control of a molluscan *(Tapes)* heart. *Comp. Biochem. Physiol.* **17,** 719—739.

SAKHAROV, D. A., T. PÉCSI (1965): Protecting and reactivating effect of serotonin on the heat-inactivated heart of the fresh-water mussel. — *Annal. Biol. Tihany* **32,** 117—121.

SALÁNKI, J. (1963): The effect of serotonin and catecholamines on the nervous control of periodic activity in fresh-water mussel *(Anodonta cygneah.).* — *Comp. Biochem. Physiol.* **8,** 163—171.

STANNARD, N. (1939): The mechanisms involved in the transfer of oxygen on frog muscle. — *Cold Spring Harbor Symposia* **7,** 394—405.

VANE, J. R., H. O. J. COLLIER, S. J. CORNE, E. MARLEY, P. B. BRADLEY (1961): Tryptamine receptors in the central nervous system. — *Nature, London* **191,** 1068—1069.

WOOLEY, D. W., B. W. GOMMI (1964): Serotonin receptors: V. Selective destruction by neuraminidase plus EDTA and reactivation with tissue lipids. *Nature, London* **202,** 1074—1075.

ADATOK A TRIPTAMINHATÁS MECHANIZMUSÁHOZ ÉDESVÍZI KAGYLÓLÁRVÁK ZÁRÓIZOMTEVÉKENYSÉGÉN

Lábos Elemér

Összefoglalás

Édesvízi kagyló *(Anodonta cygnea L.)* glochidiumainak triptaminnal kiváltott ritmikus motoros tevékenységét vizsgáltuk egyedül és különböző farmakonok jelenlétében. A ritmus modellezésére leszámlálási mechanizmust tételeztünk fel. A ritmus csillapodásának nem lehet oka indolecetsav keletkezése, mert az a triptaminhatást potencírozza.

Különböző indol-származékok vizsgálata révén a triptaminhatás specifikus voltát hangsúlyozzuk. 10^{-4} — 10^{-3} M/lit koncentrációban kisebb hatású, de hatásos indol-származék az 5-methoxytriptamin és eserin. Indol-alkilsavak, melatonin, bufotenin hatástalanok.

Az ontogenezis előrehaladtával észlelhető érzékenységcsökkenés magyarázatára az új mediátor-rendszer kialakulását tételeztük fel. Ez nem lehet kolinerg, GABA-erg, glutaminerg rendszer. Ugyanis ACh, GABA, glutamin nem gátolják a triptamin okozta aktivitást. Azonban szimpatikolitikumok alkalmazása katecholamin-gátló rendszer kialakulása mellett szól. Dibenamin és ergotamin potencírozzák a triptaminhatást. Diklórizoproterenol önmagában aktiváló és triptaminnal együtt adva potencírozó hatású. A vizsgált catecholaminok közül adrenalin, noradrenalin, IPNA potencíroz, dopamin, DOPA, α-metil-DOPA hatástalan. Így a szimpatolitikumok aktiváló hatásáért felelőssé tehető gátló catecholamin nem ezek között van.

Feltehető, hogy az ontogenezis során adrenerg mediáción alapuló gátló és aktiváló rendszerek is kialakulnak.

A NaN_3, KCN potencírozó, 2,4-DPN gátló hatásai az oxidatív anyagcserefolyamatok fontosságát hangsúlyozzák. Az azid-hatás mechanizmusát taglaltuk. A cianid-hatás a terminalis oxidáció direkt szerepére utal. Speciális inkubációs elegyben (triptamin+methionin+monojódecetsav) létrehozott fotoaktiváció ugyancsak színes (citokrom?) rendszer részvételét tételezi fel és szabad gyökös mechanizmusok fontosságát húzza alá.

Az indol-vázas vegyületekkel történő aktiválás korábbi embrionális alakok morfogenezisén is kimutatható. A triptamin itt is emelkedő hatású és 100 μg/ml koncentrációban duzzadást, dysmorphogenesist, dezintegrációt hoz létre.

К ВОПРОСУ О МЕХАНИЗМЕ ДЕЙСТВИЯ ТРИПТАМИНА НА ЗАПИРЯТЕЛЬНУЮ МЫШЦУ ГЛОХИДИЕВ БЕЗЗУБКИ

Э. Лабош

Изучали ритмическую моторику глохидиев беззубки, вызванную триптамином. Урежение ритма не связано с образованием индолуксусной кислоты.

При сравнении действия различных производных индола выяснился специфический характер влияния триптамина. Активными, хотя и в меньшей степени, были 5-метокситриптамин и эзерин. Индол-алкильные кислоты, мелатонин и буфотенин неэффективны.

Предполагается, что понижение чувствительности на более поздних стадиях онтогенеза связано с образованием новой системы медиаторов. Она не может быть холинэргической, ГАМК-эргической либо глютаминэргической. Опыты с симпатиколитиками указывают на появление тормозной системы, связанной с катехоламинами. Изопропилнорадреналин обладает замедляющим, но потенциирующим действием, дихлоризопротеренол сам обладает активирующим действием, а совместно с триптамином дает потенциирующий эффект. Это явление связано со свойствами β-адренорецепторов. Эта рецепторная система представлена в недифференцированной форме, так как эффект триптамина потенциируется и при даче антагонистов α-адренорецепторов. Из примененных катехоламинов адреналин, норадреналин и изопропилнорадреналин потенциируют, а допамин и диоксифенилаланин неэффективны. Таким образом, в это число не входит тот катехоламин, который тормозит активирующее действие симпатиколитиков.

Можно предположить, что и активирующие и тормозящие системы развиваются в онтогенезе на основе адренэргической медиации.

Влияние NaN_3, KCN, 2,4-ДНП подчеркивает значение окислительного метаболизма. Описан механизм действия азида. Влияние цианида указывает на прямое значение терминальной оксидации. Опыты с фотоактивацией, созданной в условиях специальной инкубационной среды, указывают на участие цветной (цитохромы?) системы и важность свободно-радикальных механизмов.

Активацию индольными производными можно наблюдать и на более ранних стадиях морфогенеза. Триптамин, будучи наиболее активным и на этих стадиях, вызывает нарушения развития и дезинтеграцию.