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CELL DIVISION

IV. ACCELERATION OF MITOTIC RATE OF GRASSHOPPER NEUROBLASTS BY AGMATINE

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MANY substances have been reported to inhibit mitosis, but substances that accelerate division have been found much less frequently. Among the latter are kinetin, a derivative of DNA, which stimulates cell division in plants [7] and in Yoshida sarcoma cells [8]; extracts of injured cells [5]; calcium ions [6]; sodium glucuronate [8]; and hypertonic culture medium [Gaulden, unpublished data]. Anderson [1, 2] reviewed the literature on initiation of cell division and has proposed a hypothesis implicating basic substances in stimulation of division, condensation of chromosomes [3], and formation of protein fibrils. On the basis of these considerations the basic substance agmatine (decarboxylated arginine) was selected as a possible mitotic stimulant. Agmatine was also chosen because it can be readily produced from arginine. Since Heilbrunn emphasized the role of calcium in the initiation of cell division [6], it was of interest to determine whether agmatine and calcium produced parallel effects.

MATERIAL AND METHODS

Grasshopper (*Chortophaga viridifasciata*) embryos of 14–15 days' development (26°C) were used. Each embryo was separated from its membranes and yolk in isotonic medium. The head, tip of the abdomen, and legs were removed, and the remainder of the embryo was mounted with the ventral surface against a coverslip. Approximately a quarter of the yolk of one egg was added to the small drop of medium used to cover the embryo fragment. The coverslip was inverted over a depression slide and sealed with mineral oil. The standard culture medium consisted of 6.8 g of NaCl, 0.1 g of $MgCl_2$, 0.2 g of $CaCl_2$, 0.2 g of NaH_2PO_4, 0.125 g of $NaHCO_3$, and 7.7 g of dextrose in 1 liter of glass-distilled water [4].

In the experiments in which the calcium content of the medium was altered, the NaCl content was varied to maintain isotonicity. In the calcium series, experimental

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embryos were dissected in media with altered calcium content. In the agmatine series, however, experimental embryos were dissected in control medium, then rinsed and mounted in medium containing agmatine. The experimental medium consisted of standard medium to which agmatine sulfate (Nutritional Biochemicals Corp.) had been added to a final concentration of 0.005 *M*. No change in tonicity or pH was observed from this addition.

Observations on neuroblasts were made at 22° or 38°C. Since it is known that in hypertonic and hypotonic media the duration of some stages is altered ([10], Gaulden, unpublished data), only data from preparations isotonic during the entire experimental period are included in the results. Cells in late prophase were selected for observation, and the time at which each of the following events occurred was recorded: (1) breakdown of the nuclear envelope, (2) beginning of anaphase movement of the chromosomes, (3) initiation of telophase change of the chromosomes, (4) appearance of the nucleoli, and (5) loss of spherical shape of the nucleoli. These events mark the beginning of prometa-metaphase, anaphase, and early, middle, and late telophase, respectively.

RESULTS

The effect of calcium concentration on the duration of mitotic stages is shown in Table I. An analysis of variance of the data revealed that omission of calcium from the medium resulted in a statistically significant prolongation of prometa-metaphase ($P < 0.001$) as compared to that in control medium (0.0017 *M*). The duration of anaphase, however, was not changed. With calcium concentrations greater than that of the control medium, there was also a prolongation of prometa-metaphase. For this stage, the difference

TABLE I. *Effect of calcium concentration on duration of mitotic stages.*

Temp. (°C)	Molar concn. of calcium	No. of cells	Average duration of stage (min)			
			Prometa-metaphase	Anaphase	Early telophase	Middle telophase
22	0.0000	13	40.3[a]	14.9		
	0.0017	13	26.4	15.3		
38	0.0017	9	13.0	9.0	7.0	12.7
	0.0034	2	13.5	9.0	8.0	13.0
	0.0051	9	15.8[a]	9.0	8.1	12.6
	0.0085	2	19.0	9.0		
	0.0170	1	40.0	9.0		

[a] Difference between control (0.0017 *M*) and experimental significant at 5 per cent level.

between calcium concentrations of 0.0017 and 0.0051 *M* is statistically significant at the 5 per cent level. The durations of the other stages did not differ.

The effects of agmatine on duration of some mitotic phases are given in Table II. These experiments were performed in winter and early spring. Because of the seasonal variation in respiration rates found in this material

TABLE II. *Effect of agmatine (0.005 M) on duration of mitotic stages at 38°C.*

Egg case	Treatment	No. of cells	Average duration of stage (min)				
			Prometa-metaphase	Anaphase	Early telophase	Middle telophase	Total time
I	Agmatine	10	14.4[a]	9.0	7.2[a]	12.6[a]	43.2[b]
	Control	10	25.0	9.2	11.6	16.3	58.1
II	Agmatine	5	10.0[a]	9.1	7.0[a]	14.6[a]	40.7[b]
	Control	5	13.0	8.8	9.0	20.2	51.0
III	Agmatine	10	11.4[a]	9.1	6.6[a]	18.1	45.2[b]
	Control	8	15.0	9.2	8.3	17.9	50.0
IV	Agmatine	7	10.1[a]	8.9	6.3	12.0[a]	37.3[b]
	Control	7	13.6	9.0	8.1	13.7	44.4

[a] Difference between control and experimental significant at 5 per cent level.
[b] Difference between control and experimental significant at 1 per cent level.

[11], measurements on embryos obtained from individual egg pods[1] were tabulated separately. Analysis of variance shows that, although the egg pods differed ($P < 0.01$) with respect to stage duration in the control embryos, they were similar with respect to the extent of difference between durations in control and agmatine-treated embryos. Cells in medium containing agmatine progressed faster than controls from the breakdown of the nuclear membrane to the end of middle telophase. Within this interval, it was found that the duration of prometa-metaphase was decreased by about 30 per cent, and that of early telophase by about 35 per cent. The duration of middle telophase was less than in control medium in three egg pods and did not differ significantly from the control in the fourth.

In none of the egg pods was the duration of anaphase altered; therefore, it

[1] An "egg pod" consists of the 15–30 eggs laid at one time. They are surrounded by a mucous-like substance, which when dry, holds them together as a unit. The embryos in a pod develop more or less synchronously.

was possible to determine the total number of neuroblasts that progressed through mitosis during a given period by counting the neuroblasts in anaphase at intervals equal to the duration of this stage (Table III). Preparations were made similar to those used for cell timings and a count was made every 9 minutes of all neuroblasts in anaphase in six segments (two maxillary, three thoracic, and the first abdominal segments). Since each neuroblast

TABLE III. *Effect of agmatine on total number of neuroblasts that divide during a 3-hour interval (0.005 M, 38°C).*[a]

Experimental	Control	Agmatine
1	64	111
2	59	110
3	53	76
4	79	138
5	43	110
6	73	126
Mean	62	112

[a] The difference between treatments is significant at the 0.1 per cent level. Each experiment consisted of neuroblasts of the two maxillary, three thoracic, and first abdominal segments of one control and one treated embryo.

division results in one neuroblast and a ganglion cell, the neuroblast population is constant during the experiment. In each experiment two embryos (one in control medium the other in medium containing agmatine) from the same egg pod were used. During the first 3 hours an average of 62 neuroblasts in anaphase was observed in the control preparations. In agmatine preparations an average of 112 anaphases was observed during the same period. This difference is highly significant ($P < 0.001$). An agmatine-treated embryo continued to have a mitotic rate more than twice that of the control in experiment 2 (Table III), which was prolonged to 5 hours (22 and 29 anaphases in the fourth and fifth hours as compared with 8 and 12 in the control during these periods).

Oxygen consumption was measured for two lots of embryos to determine whether the increased mitotic rate was accompanied by an increase in respiration. Oxygen consumption was measured for 1 hour; the side arms of small Warburg flasks were emptied, adding control medium to one flask and medium containing agmatine to the other; respiration was then observed for 3 hours. No difference in the rate of oxygen consumption was observed.

DISCUSSION

Direct observation of living cells has shown that agmatine accelerates the mitotic rate of part of the cycle. Since the number of cells progressing through division is increased for at least 5 hours, it may be inferred that the effect of agmatine is not limited to the portion of the mitotic cycle observed. Although the mechanism of agmatine action on the cell is still uncertain, several possibilities have been eliminated by the present work. Should agmatine act as a calcium-sparing agent, it would be expected that the effect could be stimulated by an increase in calcium concentration. Agmatine, however, accelerated the prometa-metaphase stage, whereas increased calcium concentrations retarded it. Apparently there is an optimum concentration of calcium (at or near the calcium concentration of the control medium) at which the duration of prometa-metaphase is at a minimum. There is no reason, therefore, to consider agmatine action as one of alleviation of a calcium deficiency of the control medium.

Sarkar [9] reports that versene (ethylenediaminetetraacetic acid) in concentrations between 0.0025 and 0.01 *M* caused blockage of grasshopper neuroblasts at interphase, early prophase, late prophase, and telophase. No effect was observed with concentrations below 0.01 *M* on early and middle anaphase. At higher concentrations of versene, chromosome stickiness developed and led to increased delay and blockage of mitosis. It seems likely, as suggested by Sarkar, that the effect of versene on neuroblasts is the result of calcium deprivation more severe than that produced by reducing the calcium content of the medium. Spectrophotometric studies give no evidence that versene combines with agmatine, and it therefore appears that there is no direct relation between agmatine and versene action. However, the possibility that versene binds basic substances other than calcium cannot be disregarded at present. The versene effect may result from a reduction in the amount of several basic substances available to the cell in addition to calcium.

Although it is known that respiration and mitotic rate are separable phenomena (for example, low doses of X rays that reduce the number of cells in division to zero have no demonstrable effect on respiration), increased metabolism, as occurs with increasing temperature, is accompanied by an increase in mitotic activity. The lack of an increase in oxygen consumption of embryos after the addition of agmatine to the medium, coupled with the observation that anaphase duration (which is temperature dependent) is not altered by agmatine, indicates that the effect is not the result of an over-

all increase in metabolism. Agmatine may, nevertheless, serve as an intermediate in metabolism and exerts its effect not through action as a basic substance but as a metabolite. Further studies are necessary to distinguish between the possible functions of agmatine as a metabolite or as a basic substance affecting spindle formation and chromosomal changes during mitosis.

SUMMARY

The rate at which cells pass through prometa-metaphase, early telophase, and middle telophase is accelerated by the addition of agmatine (0.005 *M*) to the culture medium. Approximately twice as many neuroblasts divide in agmatine-treated embryos as in controls during a 3-hour period. Agmatine did not act as a calcium-sparing agent since the calcium concentration of the medium was at an optimum or as a generalized metabolic stimulant. It is not possible at present to distinguish between the possible functions of agmatine as a metabolite or a basic substance promoting the chromosomal cycle and spindle formation during mitosis.

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