EFFECT OF VARIOUS STRESSES ON THE INCORPORATION OF [^{3}H]OROTIC ACID INTO GOLDFISH BRAIN RNA

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INTRODUCTION

A possible relationship of RNA metabolism to higher brain functions has been inferred from theoretical considerations[9], from experiments in which RNA-blocking antimetabolites affect behavior[2], and from reports that training affects either the rate of labeling of brain RNA by radioactive precursors[24], or changes the patterns of labeling[13]. An indication of such a qualitative change from injected labeled orotic acid has been reported in goldfish which were trained to swim upright following attachment of a styrofoam float to the lower jaw[20].

The present experiments were undertaken for two reasons — first, to establish whether there was a quantitative or qualitative alteration in the labeling of brain RNA as a result of training fish in a shock avoidance task studied extensively in this laboratory[1], and secondly, in the event that such an alteration were found, to determine whether this was a specific result of learning as distinct from more nonspecific stresses associated with training. We were also interested in whether altered labeling reflected a change in synthesis of RNA, or alternatively, in specific activities of RNA precursors. The present study reports the incorporation of intracranially (i.c.) injected [^{3}H]orotic acid into total goldfish brain RNA as well as its relative incorporation into the uridylate and cytidylate moieties (U/C ratio) of the acid-soluble fraction, as influenced by a number of behavioral and physiological parameters.

MATERIALS AND METHODS

Fish

Common goldfish *(Carassius auratus)* weighing 8.5–12 g were purchased from Ozark Fisheries (Stoutland, Mo.). Fish were not fed and were stored for approximately 1 week in 200-gal tanks and at least 24 h in individual 1.8 liter plastic home tanks prior to an experiment. Ten μl of [5-^{3}H]orotic acid (New England Nuclear, 10 μCi, 10–15 Ci/mmole) were injected intracranially (i.c.) with a Hamilton syringe fitted

to a 30 gauge needle with a plastic guard extending to within 2 mm of the tip[3]. Control and experimental fish were injected and maintained for a 4 h incorporation period at identical temperatures in aged tap water unless otherwise specified. In general, experimental values were derived from 4 to 5 groups of 2 pooled brains each.

Stresses

Mild electrical shock (0.1 sec, 3.5 V, 60 cycles, every 1.5 sec) was delivered through the water for shock-avoidance conditioning as described previously[1]. This amount of shock produces a visible twitch, but does not cause convulsions. 'Shock only' fish received this mild electrical shock. The shock was administered for 48 sec followed by 12 sec of rest for the duration of a 4 h session. Unless otherwise specified, the tanks were not aerated. In an experiment designed to test for accommodation to shock, 15-gal aerated aquaria containing 12 gal of water were employed. This shock (175 V spike peak, 1.5 sec shock interval) was supplied DC from a 12 μF capacitor. One group of 20 animals was subjected to mild shock for 18 h while a second group was stored without shocking in a similar tank. At this point all animals were injected i.c. with the labeled orotic acid, half of the animals (identified by body markings) in each tank were exchanged, and the shock was resumed for 4 additional hours.

Pentylenetetrazol (PTZ, Metrazol, Knoll Pharmaceuticals, 0.1 mg/g body weight in 10 μl of saline) was injected intraperitoneally (i.p.) twice, 5 and 35 min after the i.c. labeled orotic acid injection. Control fish received two saline injections i.p.

Aqueous carbon dioxide, bicarbonate and oxygen determinations

Carbon dioxide was determined by pH titration with a standardized NaOH solution and bicarbonate by pH titration with a standardized HCl solution. Oxygen was measured directly with an oxygen electrode (Yellow Springs Instruments Co.).

Isolation of RNA

After a given incorporation period, fish were killed and the brains were rapidly rinsed with isotonic saline and then frozen on dry ice. Two brains were generally homogenized together in 2 ml of chilled distilled water with a motor-driven Teflon pestle. Subsequent steps were performed at 0°C. The homogenate was treated with an equal volume of 10% TCA. Following centrifugation, an aliquot of the supernatant was reserved for counting and the pellet was washed successively with 2 vol. of 5% TCA, 2 vol. of 95% ethanol–10% potassium acetate, 2 vol. of 95% ethanol, and 2 vol. of ethyl ether. Kidney RNA was isolated in the same way.

Determination of U/C ratios

The dried pellet was treated for 2.5 h in 2 ml of 1 *N* KOH with stirring at 37°C. The KOH hydrolysate was then acidified with 0.4 ml of 6 *N* $HClO_4$ and centrifuged

to remove unhydrolyzed protein, DNA, and most of the $KClO_4$. An aliquot of the clear supernatant containing the RNA hydrolysate was reserved for counting, and the remainder was neutralized and evaporated with nitrogen at 40°C. The dried mixture of 2′- and 3′-nucleotide monophosphates was resuspended in 0.1 ml of carrier solution containing 5 mg/ml of each of the four 2′- and 3′-mononucleotides. Approximately 20 μl samples were subjected to electrophoresis for 40 min (Whatman No. 1 paper, 4000 V, 0.01 *M* pyridine–acetate buffer, pH 4.3). To establish labeled pyrimidine ratios, the UMP and CMP spots were identified by UV absorption, cut out and burned in a Packard Tritium Oxidizer. The ^3HOH produced was collected in Bray's scintillant[7] and counted. Counting efficiency corrections were made by means of an external standard. No sample included in the data contained less than 100 disint./min. From the ratio of radioactivities in the uridylate and cytidylate of RNA (U/C), and the ratio of radioactivities in total fish brain RNA relative to the TCA-soluble fraction (RNA/TCA), we calculated the ratio of radioactivity in RNA cytidylate relative to the total radioactivity in the TCA-soluble fraction: C/TCA = = RNA/TCA ÷ [(U/C) + 1].

Measurement of labeled precursor pools

The TCA supernatant fractions were heated at 100°C for 30 min and then extracted 4 times with 2 vol. of ethyl ether. The aqueous phase was evaporated at room temperature under nitrogen and the residue resuspended in 0.1 ml of a carrier solution containing 5 mg/ml each of 5′-UMP, 5′-CMP, uridine, uracil, cytidine and cytosine. Samples (20 μl) were subjected to electrophoretic separation and the individual compounds were located, oxidized and counted as above.

RESULTS

Distribution and variability of labeling

Four hours after an i.c. injection of 10 μCi of [^{3}H]orotic acid, an individual

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN GOLDFISH BRAIN RNA FOLLOWING INJECTION OF [^{3}H]OROTIC ACID

[5-^{3}H]Orotic acid (10 μl, 10 μCi) was injected i.c. Fish were maintained at 19.5°C and killed at times indicated. Values are expressed per fish as means ± standard deviation.

Duration	*N*	*TCA soluble fraction (disint./min)*	*RNA (disint./min)*	*RNA/TCA*	*U (disint./min)*	*C (disint./min)*	*C/TCA*	*U/C*
80 min	16	1,130,500 ±472,900	119,080 ±50,460	0.114 ±0.012	107,343 ±46,500	6,776 ±3,708	0.0067 ±0.0012	16.54 ±2.86
240 min	10	575,640 ±615,760	88,740 ±91,700	0.158 ±0.020	87,885 ±84,618	10,548 ±10,233	0.0178 ±0.0044	8.34 ±1.07

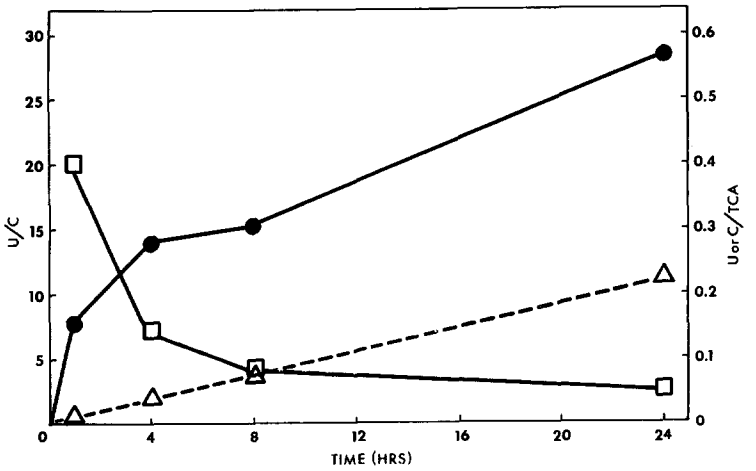

Fig. 1. The labeling of uridylate relative to TCA-soluble radioactivity, U/TCA (●-●-●); cytidylate, C/TCA (△-△-△); and the U/C labeling ratio (□-□-□) in total goldfish brain RNA at various times after the injection of [^{3}H]orotic acid.

goldfish brain typically contained 2–12 × 10^5 counts/min, of which 85% was TCA-soluble, while 15% was incorporated into RNA (see Table I). Although the amount of radioactivity in the brain varied widely from fish to fish, the fraction incorporated into RNA was relatively constant, the standard deviation being less than 15% for a group of 10 animals. Similarly, although the absolute amount of radioactive U or C found in RNA varied greatly, the ratio U/C, or U or C/TCA-soluble radioactivity showed less than 15% standard deviation for a group of 10 fish. There appeared in

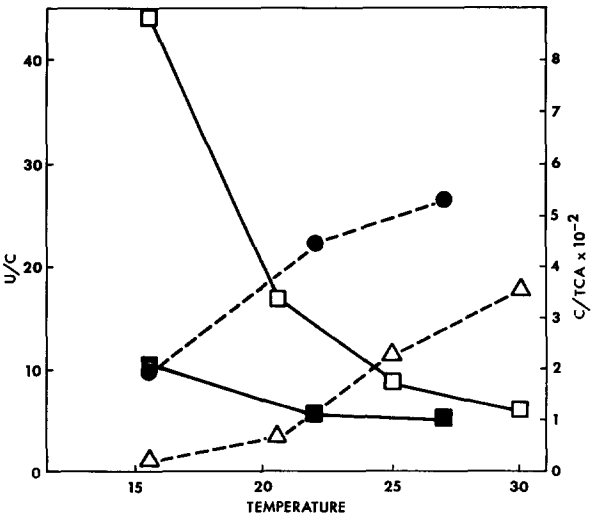

Fig. 2. The labeling of brain RNA cytidylate relative to TCA soluble radioactivity (C/TCA) and the brain RNA U/C labeling ratio at various temperatures 80 or 240 min after the i.c. injection of [^{3}H]orotic acid into goldfish. (△-△-△), C/TCA 80 min; ●-●-●, C/TCA 240 min; □-□-□, U/C 80 min; ■-■-■, U/C 240 min).

addition to be group-to-group variability, and therefore a control group was run in each experiment. Similar variabilities were seen after 80 min of labeling, although means and ratios were different as described below.

Time course of labeling and effect of temperature

The U/C ratio for total goldfish brain RNA obtained following a 4 h incorporation pulse was 7–8 at 21°C. This ratio was inversely correlated with time (Fig. 1) and temperature (Fig. 2). A 3°C increase in temperature produced a 29% decrease in the ratio for a 4 h pulse.

Pentylenetetrazol

Two sequential PTZ injections produced a marked increase in the U/C ratio, due largely to inhibition of cytidylate labeling of the RNA compared to that of the control fish, with relatively little change in uridylate labeling (Table II). We did not observe a perturbation of this ratio with ACTH injections or the styrofoam float paradigm. The RNA/TCA ratio was not significantly altered in any of the experiments in Table II, with the exception of experiment 6 in which 26 ± 4% decrease in labeling was observed (see Discussion).

Training

Four sessions (10 trials each) of shock avoidance training caused an average 25% rise in the U/C ratio in 4 out of 6 experiments. In the others, no significant change was observed. The magnitude of this change was not positively correlated with the recorded performance of fish, and as in the PTZ experiments, largely reflected a decrease in incorporation of label into the cytidylate moieties of the RNA. Mild shock alone produced a larger and more reproducible effect, averaging 46% when one fish was shocked per 1.8 liter training tank, and 149% when 4 fish were stressed in the same volume (Table II). Interestingly, if fish were subjected simultaneously to mild shock and vigorous aeration, the RNA labeling pattern was normal. With 4 fish per tank (exp. 6a), a 90% increase in U/C was observed after 1 h and the effect increased at longer times. The results suggested that crowding of fish contributed to an increase in the U/C ratio. Unshocked fish simply placed in water from a tank in which fish had been shocked showed very similar elevations in the U/C ratio. This effect was lost if the 'used' water was first vigorously aerated. The water used in this experiment was at pH 5.5 and on titration was found to contain 5 ± 1 m*M* carbonic acid ($CO_2 + H_2CO_3$), identified presumptively by its volatility and pK. Unused water contained less than 0.15 m*M* total carbonates.

Effect of carbon dioxide

To verify that the labeling effect seen in 'used' water was completely attributable

TABLE II

SUMMARY OF EFFECTS OF VARIOUS STRESSES ON GOLDFISH BRAIN RNA LABELING BY [^{3}H]OROTIC ACID

Animals were injected as in Table I. Time between injection and killing was 240 min except exp. 3 (80 min) and exp. 6 (60 min). *t*-Tests for significance were performed when a 15% or greater difference existed between experimental and control values. Equal numbers of experimental and control fish were used in each experiment.

Exp. no.	*Stress*	*N*	*Time (min)*	*RNA/TCA*			*U/C*			*C/TCA*		
				Control	*Experimental*	*% change*	*Control*	*Experimental*	*% change*	*Control*	*Experimental*	*% change*
1a	ACTH*	80	240	0.236	0.269	+14	7.17	7.70	+ 7.4	0.0289	0.0309	+ 5
2a	Float**	14	240	0.180	0.170	— 5	5.76	5.85	+ 1.6	0.0266	0.0248	— 7
2b	Float**	14	240	0.172	0.163	— 5	6.61	6.24	— 5.6	0.0226	0.0225	0
3a	PTZ***	32	80	0.114	0.122	+ 7	16.54	24.80§	+ 50	0.0067	0.0047§	—30
3b	PTZ***	20	80	0.110	0.103	— 6	21.34	51.20§	+140	0.0051	0.0022§	—57
4a	Shock condit.	20	240	0.133	0.128	— 4	5.20	6.77§	+ 30	0.0200	0.0164	—18
4b	Shock condit.	20	240	0.145	0.145	0	9.80	13.02§	+ 33	0.0134	0.0103§	—23
4c	Shock condit.	20	240	0.187	0.176	— 6	7.30	7.00	— 4.5	0.0225	0.0222	— 1
4d	Shock condit.	20	240	0.195	0.191	— 2	5.80	5.60	— 3.6	0.0287	0.0289	+ 1
4e	Shock condit.	20	240	0.157	0.138	—12	8.34	9.89	+ 19	0.0178	0.0127§	—29
4f	Shock condit.	20	240	0.149	0.134	—10	8.31	11.59§	+ 40	0.0161	0.0108§	—33
5a	Shock only,	20	240	0.178	0.173	— 3	7.91	11.79§	+ 49	0.0200	0.0135§	—33
5b	1 fish/tank	20	240	0.197	0.163	—17	6.78	9.66§	+ 42	0.0253	0.0153§	—39
6a	Shock only, 4 fish/tank	20	60	0.079	0.090	+14	14.17	27.15§	+ 92	0.0052	0.0031§	—40
7a	Shock only,	40	240	0.146	0.104§	—29	5.39	14.54§	+170	0.0229	0.0067§	—71
7b	4 fish/tank	40	240	0.170	0.141§	—27	6.78	18.08§	+167	0.0219	0.0074§	—66
7c	Shock only,	40	240	0.211	0.160§	—24	5.96	15.60§	+162	0.0303	0.0096§	—68
7d	4 fish/tank	40	240	0.208	0.155	—25	6.00	16.60§	+177	0.0297	0.0088§	—70
8a	Shock only, + aeration, 4 fish/tank	16	240	0.198	0.209	+ 6	9.56	9.49	— 0.7	0.0188	0.0199	+ 6

* 2 mg i.p. daily for 3 days preceding experiment.

** 4 h with a styrofoam float.

*** 2 injections i.p., 5 and 35 min after i.c. injection of [^{3}H]orotic acid.

§ Significant at $P < 0.01$.

TABLE III

EFFECT OF COMPOSITION OF TANK WATER ON THE INCORPORATION OF [5-^{3}H]OROTIC ACID INTO GOLDFISH BRAIN RNA

Ten fish were injected as described in Table I for each group and were killed 240 min later. Aqueous NH_3, $NaHCO_3$ or HCl were added to bring dechlorinated tap water to the stated pH.

Treatment	*pH*	*RNA/TCA*	*% change*	*U/C*	*% change*	*C/TCA*	*% change*
—	9.4	0.194	—	6.29	—	0.0266	—
NH_3	10.0	0.184	— 5	6.67	+ 6	0.0239	—10
$NaHCO_3$	8.1	0.216	+11	6.91	+ 10	0.0286	+ 8
N_2 purged	9.4	0.218	+12	7.11	+ 13	0.0270	+ 2
HCl	5.5	0.176	— 9	6.33	+ 1	0.0270	+ 2
CO_2	5.5	0.161*	—17	15.78*	+151	0.0097*	—64

* Significant at $P < 0.001$.

to carbon dioxide, a saturated carbon dioxide solution was prepared, standardized by titration, and diluted to a final concentration of 4.5 m*M* (pH 5.5). Fish placed in this water exhibited the previously observed increased U/C ratio of labeled brain RNA (Table III). Neither HCl (pH 5.5), 20 m*M* bicarbonate, ammonia (pH 10) nor oxygen-deficient (nitrogen-purged) water (44% of saturation) affected the labeling pattern in any way. In a separate experiment, acetic acid (pH 4.7) also failed to have a measurable effect.

Labeling of kidney RNA by [5-^{3}H]orotic acid

In order to establish whether the U/C labeling effect was confined to the brain, kidneys were also examined in two experiments, one in which electrical shock was administered and in another, in which PTZ convulsions were produced. In both instances, U/C was elevated in brain, but decreased in kidney. A similar inverse effect was seen in C/TCA, where the expected decrease in C/TCA in brain was accompanied by a significant increase in C/TCA in the kidney.

Shock accommodation

Fish shocked for 18 h in 15-gal aquaria before [5-^{3}H]orotic acid injection continued to show decreased cytidylate labeling in their brain RNA when compared to controls, whether or not they were shocked during the 4 h of the pulse period. The deficit was larger than that seen in fish shocked in this apparatus during the 4 h pulse period only (see Table IV). A small, statistically insignificant, increase in RNA/TCA was noted in these experiments.

Precursor pools

The partially hydrolyzed TCA-soluble fraction contained unchanged labeled orotic acid as well as 5'-CMP and 5-'UMP, representing total mono-, di- and tri-

TABLE IV

EFFECT OF ELECTRICAL SHOCK PRIOR TO AND DURING INCORPORATION OF [^{3}H]OROTIC ACID

Ten fish were used for each group. All were injected as in Table I and killed 4 h after i.c. injection of [^{3}H]orotic acid.

Electrical shock		*RNA/TCA*	*% change*	*U/C*	*% change*	*C/TCA*	*% change*
Before injection*	*After** injection*						
—	—	0.175		7.2		0.021	
—	+	0.195	+11	8.4	+17	0.020	— 5
+	—	0.186	+ 6	11.2§	+56	0.016	—24
+	+	0.196	+12	10.6§	+47	0.017	—19

* 10 h of intermittent electrical shock prior to i.c. injection.
** 4 h of intermittent electrical shock during the 4 h incorporation period.
§ Significant at $P < 0.01$.

phosphates initially present. Fractions for fish subjected to mild shock, PTZ or CO_2 contained a much higher ratio of labeled 5'-UMP to 5'-CMP than did those of control animals. As in the RNA labeling, this difference was due to a decrease in the amount of radioactivity found in 5'-CMP of the stressed fish (see Table V).

TABLE V

EFFECT OF STRESS ON THE DISTRIBUTION OF TCA-SOLUBLE RADIOACTIVITY FROM [5-^{3}H]OROTIC ACID IN GOLDFISH BRAIN

Groups consisted of 8 fish. Fish were injected as in Table I.

Group	*Incorporation time (min)*	*5'-UMP* (disint./min)*	*5'-CMP* (disint./min)*	*5'-UMP/5'-CMP*	*% change*	*U/C of RNA*	*% change*
Control	80	193,650	4,410	43.9		21.3	
PTZ**	80	156,200	1,265	123.5§	+181	51.2§	+140
Control	240	105,250	2,925	35.9		10.1	
Mild shock	240	98,250	1,175	66.7§	+ 86	20.9§	+107
Mild shock + aeration	240	92,000	3,400	27.0	— 25	9.5	— 6
Control	240	73,550	4,970	14.8		6.3	
CO_2, pH 5.5, 5 mm	240	94,250	2,940	32.1§	+117	15.7§	+149

* Total disint./min brain in 5'-nucleotide after acid hydrolysis of TCA supernatants.
** Two injections, 5 and 35 min after i.c. injection of [^{3}H]orotic acid.
§ Significant at $P < 0.01$.

DISCUSSION

There are many examples of reported quantitative or qualitative changes in brain RNA labeling seen after electrical[17], auditory[12], olfactory[18], vestibular[13] or visual[4] stimulation. Also, stress[8], anesthesia[22] and behavioral conditioning[24] have been cited as mediating increased or novel synthesis of RNA following learning (see review by Glassman[11]). Since such experiments involve the use of radioisotopes, the possibility always exists that reported alterations in RNA labeling reflect a change in *in vivo* precursor specific activities rather than a change in *de novo* RNA synthesis[5].

In the present experiments, we observed a qualitative change in RNA labeling during shock avoidance conditioning. That is, although there was little change in total RNA labeling, the pattern of labeling from [^{3}H]orotic acid was changed in a similar direction to that reported in the goldfish by Shashoua, using a different task[20]. Although it is often difficult to distinguish between effects of nonspecific stress and effects of learning, our results would appear not to reflect an effect of learning *per se*, since fish subjected to shock without conditioning exhibited similar changes.

Fish subjected to shock alone excrete increased amounts of CO_2 into their tank water, possibly reflecting higher *in vivo* P_{CO_2} levels. Interestingly, fish have been reported to increase their oxygen consumption threefold when stressed[19]. The data indicate that the increases in the U/C labeling ratio of total brain RNA reflect a decrease in the labeling of the cytidylate moieties. Fish subjected to elevated CO_2 concentrations in their tank water, whether produced by stressed fish or simply added CO_2, exhibit very similar increases in their U/C labeling ratio. Furthermore, vigorous aeration blocks the effect in shocked fish (Table II, exp. 8). These findings together support the hypothesis that an increased level of environmental CO_2, itself a result of stress, causes the perturbation in RNA labeling seen in our shocked, convulsed, or shock-conditioned fish.

Shashoua has reported that goldfish struggling to swim upright with an attached styrofoam float, show an increased U/C ratio of RNA labeling, the increase being proportional to the time necessary for mastery of the task[20]. Vigorous aeration does not, however, eliminate his labeling effect (ref. 20 and personal communication). Whether the reported increases or decreases in RNA labeling from radioactive uridine in other species can be affected by *in vivo* P_{CO_2} levels remains an interesting question[22].

In all instances, our increased RNA U/C labeling pattern is the result of a decrease in the labeling of the cytidylate moieties. Using shock, PTZ or CO_2 as the stress, a marked drop in the labeling of soluble cytidine precursors of RNA with no appreciable change in the labeling of uridine precursors suggests an inhibition in CTP synthesis or an increased degradation and efflux of cytidine metabolites. Either condition would, by lowering the specific radioactivity of this pool, increase the U/C labeling ratio of newly synthesized RNA. To conclusively establish the point would require determination of the amounts of CTP (probably less than 1 μmole/100 g tissue[15]) in the nuclei of the appropriate brain cell types at various times, a technically difficult procedure. There is, however, indirect support for this idea. Since CTP is present in much smaller amounts in tissues than the other 3 triphosphate precursors

of RNA[15], it is a likely limiting substrate for RNA polymerases. With a marked decrease in CTP synthesis and sufficient time to lower its concentration below the K_m for polymerase, RNA synthesis itself would be inhibited. We would then expect total RNA labeling to be depressed in experiments in which C/TCA was lowered for extended periods. This may explain why in Table II, exp. 7, in which C/TCA was reduced 66–71 % by the end of a 240 min period, RNA/TCA was significantly reduced (24–29 %). By contrast, in Table II, exp. 6a, the lowering of C/TCA was not accompanied by a decrease in RNA/TCA, presumably because in this 60 min experiment, the pre-existing CTP pool had not yet been exhausted.

The mechanism by which CO_2 might inhibit labeling of CTP remains unknown. However, CO_2 is known to increase cerebral blood flow in mammals[21], and could thereby change the steady-state amount of various brain substrates. The known pathway for conversion of labeled orotic acid to RNA involves the intermediate formation of labeled UTP, which may be incorporated directly into RNA or alternatively be aminated by the action of the enzyme, CTP synthetase, to form labeled CTP which is in turn incorporated into RNA. This enzyme requires glutamine and ATP. Stress and CO_2 could produce their effects at this enzymatic step. For example, cerebral supply of glutamine necessary for CTP synthesis may be lowered. Considering the concomitant increase in cytidylate labeling in kidney RNA, it is also possible that the efflux of newly synthesized brain cytidylate is increased in our experimental animals. Support for these ideas comes from experiments in which PTZ has been reported to increase cerebral blood flow[14] and others in which PTZ reduces total brain nucleotide triphosphates[23] and glutamine[10]. Electrical stimulation has also been reported to lower brain UTP and GTP[16].

In contrast to our findings, Shashoua observed no changes in the U/C ratio of the labeled RNA precursors[20]. He interpreted his labeling as indicating a qualitative change in the synthesis of RNA. The variability encountered with the small number of goldfish used, however, did not permit distinction between increases in U and decreases in C as mediators of the U/C labeling increase, precluding any conclusions regarding changes in total RNA labeling. In the present experiments, we observed no increases in RNA labeling with any of the various stresses employed. In fact, occasional decreases were seen as described above. However, a number of studies in mammals, using labeled pyrimidine nucleosides as precursors have reported increases in their incorporation into brain RNA as a function of training or stress. Bryan *et al.*[8] observed that stress causes increases in the incorporation of labeled uridine into the nuclear RNA of mouse brain. No increase in the radioactivity of the uridine precursor pool is seen. However, an increase in its specific radioactivity might be caused by an increased efflux of unlabeled uridine from the brain, since inhibition of incorporation of labeled uridine into liver RNA is seen.

Bowman and Strobel[6] observed a 26 % increase in incorporation of intravenously injected labeled cytidine into hippocampal nuclear RNA in rats undergoing training as compared to control animals, while the amount of RNA was reported to be increased 15 %. The results would support a hypothesis that some of the increase in labeling was due to an increase in specific activity of the nuclear CTP pool. In the

experiments of Zemp *et al.*[24], an increase in the specific activity of UTP from injected labeled uridine is cited as one possible explanation of an observed increase in labeling of UMP residues in mouse brain RNA. Data concerning possible increased labeling of the UTP precursor pool is not given, but a lack of increase in the labeling of low molecular weight RNA is cited as support for a hypothesis that increases in RNA labeling caused by learning reflect increases in the synthesis of messenger RNA.

While we detect no gross alterations in brain RNA labeling as a function of learning, it remains possible that minute changes in specific brain regions escape detection. These experiments serve nevertheless to exclude hypotheses that require global alterations in brain RNA as an obligatory concomitant of shock avoidance learning in the goldfish.

The effect of CO_2 on RNA labeling found in the course of these studies may be related to the report of Wasterlain[22] that rats incorporate 31% less ^{32}P into brain RNA following CO_2 anesthesia, a condition known to produce retrograde amnesia. The effect of CO_2 on RNA labeling encountered during the course of the present experiments, whether or not related to higher brain function, may nevertheless reflect a metabolic response characteristic of the nervous system.

SUMMARY

The effect of a number of stresses on the incorporation of [5-^{3}H]orotic acid into total goldfish brain RNA and its pyrimidine precursors is reported. The ratio of labeling of the uridylate and cytidylate moieties of the RNA (U/C) varied as a function of the temperature and duration of the experiment. The ratio was elevated when fish were subjected to pentylenetetrazol-induced convulsions or to mild electrical shock, with or without training of an avoidance conditioning task. The increase in ratio in all cases resulted from a decrease in cytidylate labeling. In experiments where this decrease was marked, a substantial decrease in the labeling of total RNA was seen as well. The observation that the increase in U/C was greater when increased numbers of fish were shocked per tank eventually led to the finding that addition of carbon dioxide to tank water results in a decrease in labeling of cytidylate isolated from RNA hydrolysates, as well as decreased labeling of cytidine precursors of RNA.

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