Presence of serotonin in the palate just prior to shelf elevation

ERNEST F. ZIMMERMAN, ELIZABETH L. WEE, NANCY PHILLIPS AND NANCY ROBERTS

From the Division of Cell Biology, Institute for Developmental Research, Children’s Hospital Research Foundation, Cincinnati

SUMMARY

Since serotonin and its antagonists affect shelf rotation in mouse embryo culture, experiments were carried out to determine whether a serotonergic system is present in the palate. Employing [3H]5-HT, day-14-5 embryos incorporated the monoamine into palates. Active uptake of [3H]5-HT was shown since excised palates incorporated 9-fold more radioactivity at 37 °C than at 4 °C. Synthesis of palatal serotonin was measured. Embryos were cultured in the presence of the serotonin precursor, [3H]5-HTP, and radioactive 5-HT was monitored in the palate by thin-layer chromatography. Furthermore, excised palates were incubated with [3H]5-HTP and radioactive 5-HT was measured. Incorporation was linear for about 6 h. In addition, another radioactive compound was detected which had the same Rf as the methylated derivative, 5-methoxytryptamine. Synthesis of this compound was appreciable, about 30% of that of serotonin. Levels of serotonin in the palate were measured by high pressure liquid chromatography. Palates at day 14-5 of gestation contained 0.40 ng serotonin/mg protein, which was greater than that of tongue (0.33), body (0.14) but less than that of brain (3.09). Serotonin in palate and other embryonic tissues increased with time of development. Dopamine levels in the palate and other tissues were also determined. The distribution of serotonin in the palate was analyzed by culturing day-14-5 embryos in the presence of [3H]5-HTP, and after aldehyde fixation, paraffin embedment and sectioning, autoradiography was performed. Grains were observed throughout the palate in cells of regions 2 and 3, internal mesenchyme, tooth germ, and epithelium. Surprisingly, the pterygopalatine nerve, maxillary nerve and pterygopalatine ganglion contained an appreciable concentration of grains. Thus, the presence of serotonin in the palate is consistent with the neurotransmitter playing a role in shelf elevation.

INTRODUCTION

Movement by means of the contraction of filopodia has emerged as a major means of cell locomotion during embryogenesis. Filopodial contraction has been proposed for the morphological movements associated with flask cell invagination in amphibian gastrulation (Baker, 1965), the migration of the deep cells of the teleost blastoderm (Trinkaus, 1973), chick secondary mesenchymal cell movements (Trelstad, Hay & Revel, 1967) and sea urchin gastrula-
tion (Gustafson & Wolpert, 1967). In this latter developmental process, elongated filopodial projections of secondary mesenchymal cells selectively adhere to the ectodermal roof of the blastocoel and contract to complete invagination of the archenteron. In fact, neurotransmitters appear to regulate the mesenchymal cell movements and hence gastrulation; serotonin functions early and acetylcholine functions on muscarinic receptors later (Gustafson & Toneby, 1970). The biochemical measurement and histochemical localization of serotonin appears to support its role during this process (Buznikov, Chudakova & Zvezdina, 1964; Buznikov, Sakharova, Manukhin & Markova, 1972). Specific distribution and temporal appearance of neurotransmitters such as catecholamines and serotonin have been observed during morphogenesis of the neural tube and gut (Kirby & Gilmore, 1972; Lawrence & Burden, 1973; Wallace, 1979). Evidence for the role of these neurotransmitters in morphogenesis comes from the pharmacological manipulation of the neurotransmitters leading to developmental defects of delays in the closure of the neural tube (catecholamines – Lawrence & Burden, 1973; serotonin – Palén, Thörneby & Emanuelsson, 1979) and rotation of the palate (serotonin – Wee, Babiarz, Zimmerman & Zimmerman, 1979; acetylcholine – Wee, Wolfson & Zimmerman, 1976; Wee, Phillips, Babiarz & Zimmerman, 1980).

Studies from our laboratory have suggested that palate rotation to the horizontal position may be, in part, effected by contraction of cytoplasmic processes and migration of cells, part of the non-muscle contractile systems in the palate mesenchyme and possibly the epithelium. The non-muscle contractile systems are located in two areas of the palate mesenchyme: region 2 on the tongue side extending from the top mid-palate to the posterior limit; and region 3 along the oral epithelium, extending from the middle two-thirds of the palate towards the anterior end (Zimmerman, 1979). Since serotonin has been shown to stimulate palate shelf rotation in embryo culture (Wee et al. 1979) and to stimulate mesenchymal cell movement in culture (Zimmerman, Wee, Clark & Venkatasubramanian, 1980), the presence of serotonin in the palate has been sought. It has been shown that serotonin is synthesized in the palate; the content of serotonin is measured and its distribution described. A preliminary report of this work has been presented (Zimmerman & Roberts, 1977).

MATERIALS AND METHODS

Due to light sensitivity and labile nature of serotonin and related compounds, all operations were performed using foil-wrapped containers and a darkroom facility. Standard solutions were prepared just prior to use and radioisotopes were chromatographed routinely to monitor radio and chemical decay. Only chromatographically purified radioisotopes were used in these studies. Animals. Female A/J mice (Jackson Laboratory) were mated overnight and the presence of a vaginal plug the following morning was taken as evidence of
pregnancy and designated day 0.5 of gestation. Unless otherwise stated, pregnant mice were killed by cervical dislocation at day 14.5, the time just prior to shelf rotation (Andrew, Bowen, & Zimmerman, 1973). The gravid uterus was removed immediately and placed in chilled Dulbecco’s Modified Eagle Medium (DMEM, Gibco) pregassed with 95% O₂-5% CO₂.

Embryo culture. Explantation and culture of the embryos were carried out under sterile conditions as previously described (Wee et al. 1979) except that tongues were not excised. To monitor serotonin uptake, embryos were incubated with [³H]5-HT at 37 °C for 3 h and then palate and brain excised. Alternatively, embryos were incubated with [³H]5-hydroxytryptophan ([³H]5-HTP, Amersham-Searle, 2 Ci/m-mole) for various periods of time and were monitored for their ability to convert the serotonin precursor to radioactive serotonin (5-HT). Nialamide (400 µg, Sigma) was added to 2 ml pregassed culture medium 1 h before addition of 5 µCi of [³H]5-HTP per vial, unless otherwise indicated. Embryo cultures were terminated by decapitating the heads and rinsing with three changes of chilled Dulbecco’s phosphate-buffered saline (PBS, Gibco). Palates were excised and analysed for [³H]5-HT as described below.

Tissue culture. Using sterile technique, palates were excised from day-14.5 embryos with the aid of a dissecting microscope and microforceps. Excised palates (5–10 pairs) were placed on a sterilized Millipore filter (0.22 µm, 13 mm) in the centre well of a Falcon organ culture dish. Each dish contained 1 ml of gassed culture medium with nialamide (400 µg) and was maintained at 37 °C in a CO₂ incubator (Fig. 5). After 1 h pretreatment with nialamide, 5 µCi of [³H]5-HTP was added and culture continued at 37 °C. Alternatively, 5 µCi of [³H]5-HT (New England Nuclear, 21.4 Ci/m-mole) was added and palates were incubated in the absence of nialamide as indicated in Fig. 2. Incubation was stopped by rinsing palates with three changes of chilled PBS.

Tissue extraction of serotonin. For extraction of 5-HT, embryonic tissues were homogenized in cold 80% acetone (e.g. 100 µl/palate pair) as described by Toneby (1973). However, many investigators (e.g. Maickel, Cox, Saillant & Miller, 1968) have used acidified solvents for extraction of 5-HT from adult tissues. Since preliminary experiments were carried out which demonstrated no appreciable difference between the two methods, non-acidified acetone was routinely employed. Aliquots of the homogenate were taken for protein estimation (Lowry, Rosebrough, Farr & Randall, 1951). After centrifugation at 20000 × g for 20 min, pellets and aliquots of the supernatant were counted to estimate recovery.

Thin-layer chromatography. Since overnight storage of extracts at −20 °C resulted in a lower 5-HT recovery, chromatography was immediately carried out following tissue preparation. Recovery rates were about 80% using this precaution. After blowing off the acetone at room temperature with N₂, the remaining extract was applied to a plastic-backed thin-layer chromatogram (SIL-N-HR-UV₂₅₄ with 0.2 mm MN silica gel; Brinkmann Instruments) in a
Precursors and metabolites of 5-HT used as markers (25 μg) were spotted alongside the radioactive extracts. In addition, marker 5-HT (25 μg) was added to each radioactive extract to determine exactly whether radioactivity peaks had the same mobility as marker 5-HT. Mobility of markers were monitored with u.v. light.

The solvent routinely employed for TLC was 1-propanol: NH₄OH: H₂O (8:1:1). Other solvents used were n-butanol: acetic acid: H₂O (12:3:5) and methyl acetate: isopropanol: ammonia (9:7:4).

Strips (½ cm) were cut into small pieces and radioactivity was extracted into 0.5 ml Soluene by shaking overnight at 37 °C and counted as before.

**Determination of serotonin and dopamine levels.** Palates, and other tissues were excised and immediately frozen in liquid N₂ for assay at a later time or chilled on ice and assayed immediately. No differences in monoamine levels were found and the results of both methods of collection were pooled. Adult brain was that of pregnant dams. Tissues were extracted with 25 mM HCl. Measurement of serotonin and dopamine were carried out by the method of Sasa & Blank (1977) using high-pressure liquid chromatography (HPLC) with electrochemical detection. The tissue suspension (600 μl) was homogenized with 25 μl of ascorbic acid (2-75 mg/ml), 50 μl of 0-1 M EDTA, and 50 μl of 2.4 μM 3,4-dihydroxybenzylamine hydrobromide (DHBA), which was used as an internal standard. Salt saturation of the homogenate was accomplished by adding 1 g NaCl. Three ml of butanol were added and the sample was shaken for 60 min in the cold room. The sample was centrifuged at 400 × g for 15 min and 2.5 ml of the organic phase was transferred to 4.0 ml heptane and 200 μl of 0.01 M HCl. After shaking for 10 min in the cold room, the mixture was centrifuged at 400 × g for 15 min to separate the layers; most of the butanol/heptane layer was removed and 50 μl of the aqueous layer was injected into the liquid chromatograph and analysed for serotonin and dopamine.

**Distribution of serotonin in the palate.** The localization of serotonin in the palate was sought using the histochemical technique of formaldehyde-induced fluorescence (Falck, Hillarp, Thieme & Torp, 1962). Temperature, humidity and time of heating were varied. Nevertheless, specific fluorescence of serotonin in palate could not be observed, presumably due to low concentration of monoamine in palate (Table 2). Therefore, localization of serotonin was performed by autoradiography after incorporation of [³H]5-HTP (Belenky, Chetverukhin & Polenov, 1979). Day-14-5 mouse embryos were cultured with 5 μCi of [³H]5-HTP (10⁻⁴ m final concentration) for 3 h in the presence of nialamide (400 μg/2ml) at 37 °C. Embryos were washed free of radioactive 5-HTP and incubated in radioactive-free medium for one more h. Heads were removed, washed with cold PBS and fixed for 1 h in Karnovsky's fixative at room temperature (Karnovsky, 1965) to fix serotonin or its derivatives in dense core vesicles within cells. Tongue and mandible were removed and heads were fixed in fresh Karnovsky's solution for an additional h. After paraffin embed-
Serotonin in the palate

Fig. 1. Chromatographic analysis of extracts from palate and brain after 3 h incubation of [3H]5-HT in embryo culture. Each day-14-5 embryo (5) was cultured at 37 °C in 2 ml of medium, including 1 µCi of [3H]5-HT which was mixed with non-radioactive 5-HT to make 2 x 10^-4 M. After a 3 h incubation, palate pairs and brains were excised, each tissue pooled, extracted with 80% acetone and subjected to TLC, as described in Materials and Methods.

ment, sections were coated with Kodak NTB2 emulsion and after 1–3-week incubations in the dark, slides were developed and stained with methyl green-pyronin or bismarck brown. Presence of grains were criteria of 5-HT or its derivatives in palatal cells. The results presented here are based on histological observations of six embryos from three litters and counting of grains per surface area.

RESULTS

Serotonin uptake

The first phase of the work was to determine whether foetal tissues, particularly palate, could incorporate [3H]5-HT. Embryo culture was employed to monitor serotonin uptake. As indicated in Fig. 1, a large radioactive peak was observed in both day-14-5 palate and brain which showed the same Rf as marker 5-HT (0.47). The radioactivity peak height in embryonic brain (about 200 cpm) was about 7-fold greater than a palate pair (27 cpm). However, when tissue retention of monoamine was calculated per mass, brain (42.0 n-mole 5-HT/mg protein) was only about twice that of palate (27.1 n-mole/mg). In addition, both tissues had apparently metabolized a considerable amount of the [3H]5-HT
incorporated. In palate, there was much radioactivity at the origin, the identity of which is not known. In brain, a large radioactive peak at fractions 8–9 was observed which had a mobility of HIAA, the product of monoamine oxidase. Much less radioactivity with a similar Rf was found in palate (around fraction 7). A small radioactivity peak at fractions 23–25 of both tissues was found, which comigrated with melatonin. Finally, a very small peak of radioactivity was seen in palate at fraction 17 which migrated with an Rf similar to 5-methoxytryptamine (5-MT). In contrast, brain did not show a peak of radioactivity in that area. Since both tissues not only exhibited a significant \[^3H\]5-HT peak but metabolites as well, it is unlikely that 5-HT was incorporated into only the extracellular compartment.

Since active uptake of serotonin into cells is a temperature-sensitive process, excised day-14-5 palates were incubated with \[^3H\]5-HT for various periods of time at 37 °C and 4 °C. As shown in Fig. 2, there was a 9-fold increase in 5-HT uptake into palate at 37 °C compared to 4 °C after 120 min incubation. This result indicates that palate can actively incorporate serotonin.
Serotonin synthesis

Next, experiments were carried out to test whether palate can synthesize 5-HT. Radioactive 5-HTP was incubated with the appropriate tissue and synthesis of 5-HT was monitored. Since the 5-HT uptake study (Fig. 1) showed significant metabolism of 5-HT, a monoamine oxidase inhibitor (nialamide) was added to prevent the further enzymatic conversion of 5-HT to its metabolite, 5-hydroxyindole-3-acetic acid (HIAA).

First, total embryos were cultured in the presence of [3H]5-HTP. Optimal recovery of labelled 5-HT was obtained with a 1 h preincubation with nialamide which was subsequently employed. Figure 3 shows the chromatographic separation of radioactive compounds in excised palate and brain after 18 h incorporation of [3H]5-HTP into embryos. In both tissues a radioactivity peak is seen which comigrates with marker 5-HT; the radioactivity peak was much greater in brain than in palate. To assure further that the radioactivity was actually serotonin, thin-layer chromatography of extracts labelled as before was repeated in other solvent systems. Radioactivity peaks which comigrated with marker 5-HT (as well as 5-HTP) were observed when solvents n-butanol:acetic acid: H2O and methyl:isopropanol: NH4OH were employed. Little or no HIAA was observed on these chromatograms. Figure 4 indicates that the incorporation of labelled 5-HT increases with time of embryo culture in the presence of [3H]-5-HTP. When embryos were preincubated for 1 hour with nialamide, the amount
of 5-HT recovered was consistently greater than in the absence of nialamide. Palates contained a 50% greater amount of labelled 5-HT when cultured for 18 h in the presence of nialamide.

To show directly that the palate synthesizes serotonin, excised palates were incubated with [3H]5-HTP for various periods of time. Figure 5 shows the radioactivity profile from a thin-layer chromatogram after an 18 h incorporation. A large peak of radioactivity migrated with 5-HT indicating that the palate can synthesize serotonin. Surprisingly, a radioactive peak was also found that migrated with the same mobility of 5-methoxytryptamine (5-MT).

Table 1 shows incorporation of [3H]5-HTP in excised palates for various periods of time. Serotonin and 5-methoxytryptamine peaks were integrated and cpm per µg palatal protein calculated. Serotonin was progressively synthesized for 6 h; synthesis at 18 h tailed off. 5-Methoxytryptamine, which was also proportionally synthesized with time to 6 h, corresponded to a significant portion of serotonin. Synthesis of 5-MT was about 30% of 5-HT. The high
Fig. 5. Chromatographic separation of labelled 5-HT after an 18 h incorporation of \([^3H]5\)-HTP into cultured excised palates. Palate pairs (8) were placed on a Millipore filter, overlaid on a wire grid in a small Petri dish containing 1 ml of tissue culture medium. Palates were preincubated for 1 hr at 37 °C in 5% CO₂ with nialamide (400 µg/ml). \([^3H]5\)-HTP (2-5 µCi) was added and incubated for 18 hr. The pooled palates were analysed for 5-HT as before.

Table 1. Incorporation of \([^3H]5\)-HTP into excised palates

<table>
<thead>
<tr>
<th>Time-h</th>
<th>n</th>
<th>cmp/µg protein</th>
<th>5-HT</th>
<th>5-MT</th>
<th>5-MT × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>3</td>
<td>0-24</td>
<td>0-24</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1-71</td>
<td>0-62</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2-41</td>
<td>0-69</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>4-30</td>
<td>1-12</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>4-71</td>
<td>1-47</td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>

Values are average cmp/µg protein from number of replicate experiments indicated by n.
Table 2. Content of serotonin and dopamine in various tissues during development

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day of development</th>
<th>5-HT ng/mg protein</th>
<th>Dopamine ng/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palate</td>
<td>14-5</td>
<td>0-40 ±0-04</td>
<td>1-63 ±0-25</td>
</tr>
<tr>
<td></td>
<td>15-5</td>
<td>0-58 ±0-06</td>
<td>1-00 ±0-09</td>
</tr>
<tr>
<td>Tongue</td>
<td>14-5</td>
<td>0-33 ±0-08</td>
<td>1-84 ±0-26</td>
</tr>
<tr>
<td></td>
<td>15-5</td>
<td>0-81 ±0-23</td>
<td>1-77 ±1-12</td>
</tr>
<tr>
<td>Bodies</td>
<td>14-5</td>
<td>0-14 ±0-04</td>
<td>1-00 ±0-12</td>
</tr>
<tr>
<td>Brain</td>
<td>14-5</td>
<td>3-09 ±0-20</td>
<td>2-38 ±0-64</td>
</tr>
<tr>
<td></td>
<td>15-5</td>
<td>4-25 ±0-33</td>
<td>3-23 ±0-51</td>
</tr>
<tr>
<td>Brain</td>
<td>Adult</td>
<td>26-4 ±5-43</td>
<td>25-0 ±4-98</td>
</tr>
</tbody>
</table>

Values presented are means ± s.e. Significance determined with 2-tailed Student's t-test

relative value of 5-MT at 0-5 h may be inaccurate because of low peak height values of 5-HT and 5-MT in relation to background values.

Content of serotonin and dopamine

Next, a direct analysis of serotonin in the palate was carried out. Excised palates and other tissues were extracted and assayed for serotonin by high-pressure liquid chromatography. Serotonin levels in the palate at day 14-5 of gestation were low. This necessitated using pooled samples of 80–100 palate pairs to obtain adequate peak heights for measurement. For day-15-5 embryos, 30–50 palate pairs were employed. The mean values presented are from four to seven separate determinations; day-14-5 palate represents seven determinations.

As Table 2 shows, the level of serotonin at day 14-5 of development is 0-40
Serotonin in the palate

ng/mg protein, which is significantly greater than that of fetal tongue at that time of development: 0.33 ng/mg, $P < 0.05$. Moreover, serotonin was found in the day-14-5 fetal body (minus head) at 0.14 ng/mg, significantly less than that of palate ($P < 0.002$). In contrast, embryonic brain has about 8-fold greater serotonin than the palate: 3.09 ng/mg, Thus, serotonin is distributed unequally in the embryo; more in the head than the body, but less in the palate than the brain (as would be expected). It was of interest to determine if palate serotonin levels were highest at the time of rotation. This was not the case, serotonin levels increased in the palate at day 15-5 of development. Serotonin levels also increased in embryonic brain at the later developmental time. Adult brain levels were 8.5-fold greater than day-14-5 brain.

The HPLC method employed could simultaneously measure dopamine levels and these analyses were also carried out (Table 2). Thus dopamine was present in the day-14-5 palate at 1.63 ng/mg protein, about 4-fold greater than that of serotonin. Dopamine levels decreased in the day-15-5 palate, and dopamine was present in the embryonic body in lower concentrations than the palate at day 14-5 of gestation. Dopamine levels increased in brain with time of development.

Distribution of serotonin

Since serotonin was present and synthesized in the palate, its distribution in the palate was sought. To observe the localization of serotonin in the palate when present in very low levels, autoradiography was employed after $[^3H]5$-HTP incorporation. Stained paraffin sections showed grains in cells throughout the palate. Region-3 cells (Fig. 6D) which possess many filopodial processes did not contain an increased concentration of grains compared to internal mesenchymal cells (Fig. 7 C). Similarly region-2 cells (Fig. 6C), and tooth-germ epithelium or its mesenchyme (not shown) also contain many grains but their concentration was not higher than other palatal structures. In addition, grains could be observed in the epithelium (Fig. 7 C). Surprisingly the pterygopalatine nerve and ganglion showed the presence of grains (Fig. 7 B). As Fig. 6B indicates,

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Fig. 7. Autoradiography of embryonic palate and eye. (A) Slightly more posterior palate section than Fig. 6 indicating region 2, pterygopalatine ganglion (G), pterygopalatine nerve (PN) and oral epithelium (OE). Bar, 75 $\mu$m. $\times 135$. (B) Higher magnification of area of ganglion and nerve. Much higher concentration of grains are observed in pterygopalatine nerve (PN) and ganglion (G) than in surrounding mesenchymal cells. Bar, 10 $\mu$m. $\times 860$. (C) Lower area of oral side of palate shelf. Grains can be seen in pterygopalatine nerve (PN) and ganglion (G) than in surrounding mesenchymal cells. Bar, 20 $\mu$m. $\times 450$. (D) Section of eye from day-14-5 embryo showing grains throughout the eye, including less (L). Bar, 15 $\mu$m. $\times 670$. (E) Higher magnification of area of eye depicted in square of Fig. 7D. Grains appear to be localized at junction of inner and outer layer of the optic cup (arrow) as well as in the outer layer. Bar, 15 $\mu$m. $\times 720$. 
many grains were localized also in the maxillary nerve. In this palate, the pterygopalatine nerve was found in a slightly more posterior section (Fig. 7A). Thus grains appeared throughout the palate including palatal neural tissue.

Other areas of the head including brain were also observed for the presence of grains. They were found throughout the brain, but were found in highest concentration in the eye (Fig. 7D). As seen in Fig. 7E, grains were localized at the junction of inner and outer layer of the optic cup and in the outer layer which is destined to become the retina. Accumulation of indoleamines in the developing retina has been reported previously (Baker & Quay, 1969; Ehinger & Florén, 1978).

**DISCUSSION**

The experiments carried out in this study were to determine whether serotonin was present and synthesized in the palate since serotonin appeared to regulate palate shelf rotation (Wee et al. 1979). Evidence that a serotonergic system exists in the palate is: (1) Serotonin was actively taken up by excised palates. (2) Serotonin metabolites were found in the palate. (3) Synthesized serotonin was located in the palate when embryos or excised palates were cultured with [3H]5-hydroxytryptophan. (4) Serotonin was detected in excised palates.

Active uptake of serotonin into neurons has been a noted feature of serotonergic systems. This active uptake has been identified by its temperature dependency. Presumably the cellular uptake process is a method of inactivating serotonin after its release and subsequent binding to its receptor. Excised palates when incubated with [3H]5-HT also showed a temperature-sensitive uptake; 9-fold more radioactivity was associated with palate incubated at 37 °C than at 4 °C (Fig. 2), constituting a criterion for a palatal serotonergic system.

After uptake into cells, serotonin is usually metabolized by monoamine oxidase to 5-hydroxyindoleacetic acid. When embryos were cultured with labelled 5-HT, little HIAA was observed in the palate compared to embryonic brain (Fig. 1). However, incubating embryos with the monoamine oxidase inhibitor nialamide increased the concentration of serotonin synthesized in the palate (Fig. 4) suggesting that some monoamine oxidase is present in the palate. This would seem likely since rat brain at day 15 of gestation had 10% of the adult monoamine oxidase activity (Saavedra, Coyle & Axelrod, 1974), while monoamine oxidase activity towards serotonin in chick retina also increased during development (Suzuki, Noguchi & Yagi, 1977). Low monoamine oxidase activity in developing palate could allow serotonin to be released from its sources and diffuse a considerable distance while acting on palate mesenchymal cells before it was enzymatically inactivated.

Labelled serotonin was observed in the palate when the precursor [3H]5-HTP was incubated with whole embryos (Fig. 3). Since brain (Baker & Quay, 1969) or yolk granules in early Ophryotrocha embryos (Emanuelsson, 1974) are sources of serotonin, it is possible that synthesized serotonin was transported
into the palate from other sites of synthesis via the functioning cardiovascular system. However, excised palates were also able to convert $[^3H]$5-HTP to 5-HT indicating that synthesis could take place in the palate directly (Fig. 5). Nevertheless, there has been some controversy on the specificity of the enzyme, amino acid decarboxylase, that converts the precursor to the monoamine (Lovenberg, Weissbach & Udenfriend, 1962; Sims & Bloom, 1973).

In any case, the presence of serotonin in the palate has been ascertained by direct measurement using HPLC. The level of serotonin in the palate at day 14-5 of development is 0.40 ng/mg protein which is significantly greater than that of the fetal tongue and body at the same time of development. This greater palatal level of serotonin is consistent with a regulatory role in the palate. However, palatal serotonin is about 8-fold less than that in brain at this time in development. In turn, the adult brain level (26.4 ng/mg) is 8.5-fold greater than brain serotonin at day 14-5 of development. Previously, Saavedra (1977) has reported a heterogenous distribution of serotonin in discrete areas of adult rat brain ranging from 2.0 ng/mg protein in the cerebellum to 36.4 in the nucleus arcuatis of the hypothalamus. Developmental increases in serotonin have also been reported previously. Kato (1960) indicated that serotonin content increased about 5-fold from the fetus to the adult in rat brain, while Bourgoin, et al. (1977) showed a 3-fold increase from the neonatal to the adult stage. The developmental increase in brain serotonin levels is also mimicked in the palate which is significantly elevated at day 15-5 of development compared to one day earlier (Table 2).

In addition, dopamine levels in the palate were measured which are much greater than that of serotonin. At day 14-5 of development dopamine levels are 4-fold more than serotonin. However, dopamine as well as norepinephrine have been tested for their ability to affect palate shelf rotation in embryo culture. In contrast to serotonin or acetylcholine, these catecholamines did not stimulate palate rotation employing concentrations as high as $10^{-4}$ M (Zimmerman et al. 1980). It may be that these catecholamines are involved in regulating palatal fusion via the cyclic AMP system; activation of adenylate cyclase (Waterman, Palmer, Palmer & Palmer, 1976) would then elevate cyclic nucleotide which in turn would cause programmed cell death and contribute to the fusion process (Greene & Pratt, 1979).

Since region-3 cells have been implicated in palate reorientation (Zimmerman, 1979), it was expected that serotonin might be preferentially localized in the area of region-3 cells. However, when autoradiography of sectioned palate was performed after incorporation of $[^3H]$5-HTP in the embryo and aldehyde fixation, grains (5-HT or metabolites) were found throughout the palate shelf. Structures such as region-2 cells, tooth germ, internal mesenchymal cells, and epithelium all showed many grains. In addition, grains were observed also in the pterygopalatine ganglion, maxillary nerve and the pterygopalatine nerve. The presence of grains in the pterygopalatine nerve (Fig. 7B) was surprising
since this nerve is sensory, although there may also be parasympathetic innervation as well. However, electron microscopic analysis of the palate revealed many dense core vesicles in this nerve (Kuhn, Babiarz, Lessard & Zimmerman, 1980) which is consistent with serotonin or catecholamine localization. One possibility to explain the presence of grains in the pterygopalatine nerve is that serotonin is localized in the nerve at day 14·5 of gestation, and at a later time in development when the nerve assumes its sensory function, the neurotransmitter composition would change. The concept that neurotransmitter composition in neurons is developmentally altered as the surrounding target field changes has been postulated by Bunge, Johnson & Ross (1978). Alternatively, the deep petrosal nerve which is sympathetic could be coursing through the pterygopalatine nerve to innervate blood vessels. At this time in development the nerve trunk which courses down into the palate lies adjacent to the palatine artery. Thus uptake of 

\[ ^{3}\text{H} \] \text{5-HTP} by sympathetic components in the nerve could convert the precursor to serotonin by the relatively non-specific amino acid decarboxylase.

The presence of 5-methoxytryptamine in the palate (Table 1) is of interest because it has been reported to be the active indoleamine neurotransmitter in sea urchin development (Renaud, Parisi, Capasso & Monroy, 1979). A function for palate 5-methoxytryptamine is not known at present.

In summary, serotonin has been shown to be present in the palate at the time of its elevation which is consistent with the monoamine regulating the morphogenetic process. Alternatively, serotonin may be regulating growth or differentiation of the palate since it has been postulated to regulate these processes in sea urchin (Buznikov et al. 1972) and mammalian neural development (Vernadakis & Gibson, 1974; Lauder & Krebs, 1978). However, a role for regulating palate morphogenesis is suggested by the following effects of serotonin: (1) Stimulation of palate shelf rotation in embryo culture; and blockade of rotation by its antagonists (Wee et al. 1979). (2) Stimulation of contractility of palate cells growing in explants (Zimmerman, 1979). (3) Stimulation of palate cell migration in explants in a collagen gel matrix and cultured cells undergoing chemotaxis (Zimmerman et al. 1980). (4) Stimulation of palatal protein carboxymethylation (Zimmerman et al. 1980), a process associated with cell migration (O'Dea, et al. 1978).

The presence of serotonin in palatal neural tissues suggest that these structures could be playing a key role in palate rotation, possibly as a source of extracellular serotonin; nerves have been observed to be active at this time of development (Wragg, Smith & Borden, 1972). The serotonin released from neural tissue could serve to initiate the secretion of serotonin by mesenchymal and epithelial cells, and thus act as a trigger for palate rotation.

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