INFLUENCE OF PSORALEN ON MELATONIN FORMATION IN RAT PINEAL GLAND IN ORGAN CULTURE

By

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ABSTRACT

8-Methoxypsoralen (10^{-4} M) added to the medium of rat pineal glands in organ culture induces a five-fold increase of 14C-labelled N-acetylserotonin and melatonin formation when serotonin is used as 14C-labelled precursor. Addition of d-amphetamine (10^{-5} M) to pineal glands also results in an increase of N-acetylserotonin and melatonin formation. This increase is enhanced by the addition of 8-methoxypsoralen (10^{-5} M).

In pineal glands pre-incubated for 24 h 8-methoxypsoralen (10^{-4} M) caused an increased production of N-acetylserotonin and melatonin. This indicates that the effect of 8-methoxypsoralen is mediated through a post-synaptic event and not by release of noradrenaline or blocked re-uptake of amines to nerve terminals in the pineal gland. Further studies of the clinical significance of the effect of psoralen on pineal gland metabolism seems warranted.

Psoralens in combination with ultraviolet irradiation have been reported to exert a beneficial effect on psoriatic skin disorders both when applied locally (Allyn 1973; Mortazawi & Oberste-Lehn 1973; Tronnier & Schäle 1973; Walter & Voorhees 1973; Walter et al. 1973; Weber 1974) or ingested per os (Oddoze et al. 1967; Parrish et al. 1974; Swanbeck et al. 1975). 8-Methoxypsoralen (6,7-furocoumarin, methoxsalen, meloxine) (Fig. 1) as opposed to trimethylpsoralen has been reported to be effective in the systemic treatment of psoriasis (Swanbeck et al. 1975). The amelioration of skin symptoms is thought to be primarily caused by diminished DNA-replication in the epidermal cells (Baden et al. 1972; Cole 1970; Epstein & Fukuyama 1970; Walter et al. 1973). In the rapidly
dividing epithelium of the psoriatic plaque a decreased level of cyclic AMP (adenosine 3',5'-monophosphate) has been found in vivo (Voorhees et al. 1972a). In vitro studies have revealed that administration of cyclic AMP inhibits epidermal cell division (Voorhees et al. 1972b) and a disturbed balance in the turnover rates of intra-cellular cyclic nucleotides causing psoriatic disturbances has been suggested (Voorhees et al. 1973a,b). A side effect of psoralen treatment in psoriasis is the occurrence of hyperpigmentation in the area treated with topical psoralen (Mortazawi & Oberste-Lehn 1973; Walter & Voorhees 1973; Walter et al. 1973) or diffuse pigmentation following oral psoralen (Parrish et al. 1974) and exposure to ultraviolet light. Psoralens occur naturally in several oriental plants and have been administered topically (Jarrett & Szabó 1956; Kanof 1955; Kelly & Pinkus 1955) or systemically (Elliott 1956, 1959; Lerner 1953; Zimmerman 1959) to stimulate re-pigmentation of skin areas affected by vitiligo (Fitzpatrick & Pathak 1959). In general, skin-pigmentation control is mainly dependent on enzymatic turn-over of the amount of pigment within the melanocyte and factors that affect the movement and distribution of melanin granules (Lerner & Case 1959).

Melatonin, the specific pineal gland hormone is at present the most potent agent known to bleach amphibian skin through aggregation of melanin granules (Burgers & van Oordt 1962; Lerner et al. 1958; Lerner & Case 1959). MSH (melanocyte-stimulating hormone) and ACTH (adrenocorticotrophic hormone) counteract this effect, that is they darken frog skin dermal melanocytes (Lerner & Case 1959; Novales 1963). MSH and ACTH were shown to mediate their melanin dispersion through an increased cellular level of cAMP (Abe et al. 1969a,b; Bittensky & Demopoulos 1970). Melatonin counteracts skin darkening through inhibition of cAMP increase (Abe et al. 1969a). No direct effect of melatonin has been accurately demonstrated on melanin aggregation in epidermal melanocytes in amphibians (McGuire & Möller 1966), man (McGuire & Möller 1966) and other mammals (Snell 1965). However, in the last few years several reports have supported the existence of the pineal-hypothalamic-
pituatory axis as a route for pineal influence on pigmentation control. Activation of rat pineal gland by darkness or injecting the rat with melatonin intraperitoneally decreases pituitary MSH-activity (Kastin et al. 1969; Kastin & Schally 1967). Conversely, depression of the pineal gland function either by surgical removal of the gland or by exposing rats to constant lighting (Wurtman et al. 1963) increases MSH-activity in the pituitary (Kastin et al. 1967a,b). Pinealectomy or melatonin administration has been reported to enhance or slow down hair wave cycles in mice (Houssay et al. 1966). Pinealectomized rats have been shown to exhibit greater erythematous skin reactions than controls following exposure to ultraviolet light (Mori et al. 1968). A case report has been described showing that a 2% melatonin ointment applied to the skin of patients treated with psoralens and shortwave irradiation prevented undesirable hyperpigmentation (Izawa 1967).

In view of the data indicating the possibility that pineal products and psoralens interact in the control of skin function it was considered interesting to see whether 8-methoxypsoralen could directly affect pineal gland metabolism.

**Materials and Methods**

Pineal glands were taken from male Sprague-Dawley rats (225–250 g). The animals were kept on a 12:12 h light:dark schedule with lights on between 6 a.m. and 6 p.m. The rats were kept five to a cage and fed water and food pellets *ad libitum*. They were kept under these conditions for a period of 7–10 days before the experiment. The rats were sacrificed by decapitation between 10–12 a.m. Pineals were rapidly dissected out and stored for 1–3 min in icecold culture medium before being introduced into the culture chamber.

The pineal organ culture method has been described elsewhere but is basically a technique developed by Trowell (1959). For further details see Bäckström & Wetterberg (1973).

The medium was supplied with 0.25 mM [14C]serotonin creatinine sulphate (4 mCi/mmole). Pineals were pre-incubated for 1–1.5 h before the drugs were added. In one group 8-methoxypsoralen was added 30 min before the following drug, i.e. d-amphetamine, was introduced.

In another group, pineal glands were pre-incubated for 24 h in the medium without isotope and then transferred to fresh media, containing [14C]serotonin to which the drugs were added.

After 24 h of incubation following the addition of drugs, [14C]-labelled N-acetylserytonin (NAcS), melatonin (MEL) and 5-hydroxyindoleacetic acid (5-HIAA) in the media were isolated by thin-layer chromatography (Klein & Notides 1969) and then eluted. Radioactivity was measured in a Packard Tri-Carb Scintillation Spectrometer Model 3380, supplied with an Absolute Activity Analyzer Model 544 using Instagel® or Aquasol® for counting solutes.

Student’s *t*-test was used for statistical analyses. Data in the tables are presented as mean ± standard error of the mean (SEM) of picomoles of metabolites in culture medium formed per 24 h per pineal gland.
Table 1.
Production of serotonin (5-HT) derivatives by cultured pineal glands from [14C]5-HT.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. in medium (m)</th>
<th>N</th>
<th>Metabolites in medium</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-Acetyl-5-HT</td>
<td>Melatonin</td>
<td>5-HIAA</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>4</td>
<td>235 ± 43</td>
<td>240 ± 18</td>
<td>3.246 ± 463</td>
<td></td>
</tr>
<tr>
<td>8-Methoxypsoralen 10^-4</td>
<td></td>
<td>3</td>
<td>1.191 ± 129***</td>
<td>870 ± 64**</td>
<td>2.281 ± 376</td>
<td></td>
</tr>
<tr>
<td>8-Methoxypsoralen 10^-5</td>
<td></td>
<td>4</td>
<td>385 ± 44</td>
<td>508 ± 169</td>
<td>2.861 ± 580</td>
<td></td>
</tr>
<tr>
<td>8-Methoxypsoralen + d-Amphetamine 10^-5</td>
<td></td>
<td>4</td>
<td>2.524 ± 152***</td>
<td>910 ± 71***</td>
<td>2.369 ± 957</td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine 10^-5</td>
<td></td>
<td>3</td>
<td>1.463 ± 123***</td>
<td>1.055 ± 56***</td>
<td>2.489 ± 12</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± sem of picomoles formed per 24 h incubation period.

*** designates groups that differ significantly in amounts of metabolites found from [14C]5-HT from control value = P < 0.001.

N-Acetyl-5-HT = N-acetylserotonin.

5-HIAA = 5-hydroxyindoleacetic acid.

N indicates the number of pineal glands assayed.

Chemicals

5-Hydroxy (side chain-2-[14C])tryptamine creatinine sulphate, [14C]serotonin 54 mCi/mmol (Radiochemical Centre, Amersham); 5-hydroxytryptamine (serotonin) creatinine sulphate (Fluka AG); d-amphetamine sulphate (Siegfried AG); 8-methoxypsoralen (Torkel Fischer, Uppsala); 1-noradrenaline bitartrate (Sigma Chem. Co.).

Concentrations are given as concentrations of the salt. Drugs were weighed and diluted immediately before the experiment and kept in darkness at +4°C until used.

d-Amphetamine was dissolved in distilled water, 1-noradrenaline in 0.01 m HCl and 8-methoxypsoralen in 80% ethanol. 5 μl of drug solution was added to 0.6 ml of organ culture medium.

RESULTS

In pineal glands pre-incubated for 1–1.5 h (Table 1) d-amphetamine 10^-5 m, and 8-methoxypsoralen 10^-4 m caused significant increases in the formation of [14C]NAcS and [14C]MEL (P < 0.001) but no significant changes in the formation of [14C]5-HIAA. 8-Methoxypsoralen 10^-5 m by itself could not be shown to cause any change in the metabolite production but significantly potentiated the stimulant effect of d-amphetamine 10^-5 m addition on NAcS formation (P < 0.01).
Table 2.
Production of serotonin (5-HT) derivatives by cultured pineal glands from [14C]5-HT. All pineal glands were pre-incubated 24 h and then transferred to culture media containing [14C]5-HT.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. in medium (M)</th>
<th>N</th>
<th>Metabolites in medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-Acetyl-5-HT</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td></td>
<td>196 ± 18</td>
</tr>
<tr>
<td>Ethanol</td>
<td>6 %o</td>
<td>5</td>
<td>239 ± 62</td>
</tr>
<tr>
<td>8-Methoxypsoralen 10⁻⁴</td>
<td>5</td>
<td></td>
<td>743 ± 66* * *</td>
</tr>
<tr>
<td>8-Methoxypsoralen 10⁻³</td>
<td>5</td>
<td></td>
<td>239 ± 29</td>
</tr>
<tr>
<td>d-Amphetamine 10⁻⁵</td>
<td>4</td>
<td></td>
<td>190 ± 40</td>
</tr>
<tr>
<td>l-Noradrenaline 10⁻⁵</td>
<td>4</td>
<td></td>
<td>1.147 ± 87* * *</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± sem of picomoles formed per 24 h incubation period.

*** designates groups that differ significantly in amounts of metabolites found from [14C]5-HT from control value = P < 0.001.

N-Acetyl-51HT = N-acetylsertotonin.

5-HIAA = 5-hydroxyindoleacetic acid.

N indicates the number of pineal glands assayed.

In pineal glands pre-incubated for 24–24.5 h (Table 2) d-amphetamine 10⁻⁵ M, did not change the formation of 14C-labelled metabolites as compared to controls. l-Noradrenaline 10⁻⁵ M and 8-methoxypsoralen 10⁻⁴ M also present in these pineal cultures caused an increased production of [14C]NAcS and [14C]MEL (P < 0.001). 5-HIAA levels were in no case found to be statistically different from controls.

Ethanol 6 %o and 8-methoxypsoralen 10⁻⁵ M did not significantly affect the 14C-labelled serotonin metabolite formation.

DISCUSSION

It was found that 8-methoxypsoralen 10⁻⁴ M increased the formation of [14C]NAcS and [14C]MEL from [14C]serotonin in rat pineal gland organ culture. The stimulant effect of this psoralen on the production of [14C]NAcS and [14C]MEL did not require the presence of endogenous noradrenaline. This was shown using pineal glands pre-incubated for 24 h. These pineals do not
contain enough transmitter (Bäckström & Wetterberg 1973) to produce stimulant effect on indole metabolism following the addition of the potent amine releaser, d-amphetamine 10^{-5} M (Boakes et al. 1972; Carlsson 1970; Carr & Moore 1969; Ziance et al. 1972). As [^{14}C]5-HIAA levels were not significantly changed the effect does not seem to be mainly mediated via inhibition of MAO (monoamine oxidase) as seen following harmine (10^{-5} M) treatment (Klein & Weller 1970).

The psoralen was diluted in 80% ethanol to a final concentration of 6% ethanol (v/v) in the culture medium. This concentration of ethanol did not by itself change the formation of ^{14}C-labelled metabolites as compared to controls. Tytell & Myers (1973) reported that ethanol caused increased conversion of [^{14}C]serotonin to [^{14}C]5-HIAA in rat cerebral tissue. Any stimulant effect of ethanol on [^{14}C]5-HIAA formation could not be shown to be statistically significantly in the present experiments.

The NAcS, and a concomitant MEL increase in rat pineal gland organ cultures is mediated through stimulation of noradrenergic beta-receptors (Shein 1971), causing stimulation of adenylcyclase activity (Weiss & Costa 1967) and cAMP formation (Klein et al. 1970). Inhibition of the cAMP metabolizing enzyme phosphodiesterase by theophyllamine (10^{-3} M) (Klein & Berg 1970) or addition of the specific MAO inhibitor clorgyline (10^{-6} M) (Johnston 1968) has a similar effect like that of adrenergic beta-stimulation. The noradrenaline induced cAMP increase activates NAT (N-acetyltransferase) (Deguchi & Axelrod 1972) enzyme through a mechanism that requires differential gene transcription and protein synthesis (Fontana & Lovenberg 1973; Romero et al. 1975). It has not been possible in the present experiments to determine where in this series of events 8-methoxypsoralen interacts.

A high concentration (10^{-4} M) in the culture medium of 8-methoxypsoralen is required to affect the yield of [^{14}C]serotonin metabolites. Psoralens are poorly water soluble and it cannot be excluded that the receptors encountered a lower concentration of the drug than indicated.

The psoralen effect is probably exerted post-synaptically in the pinealocytes and not mediated by release of noradrenaline or block of the re-uptake of amine to nerve terminals in the gland, as the effect persisted in noradrenaline depleted pineal glands (Table 2). The synergistic effect of 8-methoxypsoralen (10^{-5} M) and d-amphetamine (10^{-5} M) (Table 1) also suggests different mechanisms of action for the two drugs.

The clinical significance of the present findings is not clear but further studies concerning the effects of psoralens on pineal gland metabolism seems warranted. These should include experiments on the possible role of psoralens on the anti-gonadotrophic effects of melatonin-free factors of the epiphysis (Orts et al. 1976) as well as their effects via the pineal on the adrenal and thyroid glands (DeFronzo & Roth 1972).
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REFERENCES

Voorhees J., Kelsey W., Stawiski M., Smith E., Duell E., Haddox M. & Goldberg N.

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