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EFFECT OF PSYCHOTROPIC DRUGS ON [^{14}C]TRYPTOPHAN METABOLISM IN CULTURED RAT PINEAL GLAND

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Abstract—A potentiated production of [^{14}C]melatonin ([^{14}C]Mt) with a simultaneous decline in [^{14}C]serotonin ([^{14}C]5-HT) conversion to its final metabolite [^{14}C]5-hydroxyindole acetic acid ([^{14}C]5-HIAA) was recorded in a rat pineal medium cultured with [^{14}C]tryptophan and either a neuroleptic [haloperidol (HAL) or chloropromazine (CPZ)] or an antidepressant [desipramine (DIP)] drug. A difference between the pharmacological groups of drugs was evident only in [^{14}C]5-HT levels, which were found significantly elevated in the presence of the neuroleptic HAL and reduced in the presence of the tricyclic antidepressant DIP. A parallel trend was observed in norepinephrine (NE)-induced [^{14}C]tryptophan metabolism in the pineal culture medium. All three drugs tested brought about further enhancement of NE-induced synthesis of MT; HAL produced an elevated, but DIP a reduced, level of [^{14}C]5-HT, compared to the NE-induced value.

The effects of neuroleptic drugs on the synthesis of melatonin (MT), which is one of several biologically active constituents of the pineal gland, have been studied by a number of researchers. Chlorpromazine (CPZ) was reported by Smith *et al.* [1] to increase serum MT in psychiatric patients. They concluded that CPZ reduces the rate of MT metabolism in the body, an opinion shared by Wurtman's group [2, 3], which had previously suggested this as an explanation for a similar increase in rats. However, other neuroleptic drugs, haloperidol (HAL) and fluphenazine, have been found by Hartley *et al.* [4] to inhibit *in vitro* methoxylation of *N*-acetylserotonin (NAS) by hydroxyindole-*O*-methyltransferase (HIOMT) and, thus, to have an inhibitory effect on MT synthesis.

We decided to study the effects of both types of neuroleptics—a phenothiazine, CPZ, and a butyrophenone compound, haloperidol (HAL)—on serotonin (5-HT) and MT synthesis and turnover, in a culture medium incubated with their precursor, [^{14}C]tryptophan. The antidepressant drug desipramine (DIP) was used for comparison. Parfitt and Klein [5, 6] found that DIP increased MT production from tryptophan *in vitro* [5], as well as accelerated serotonin-*N*-acetyltransferase (SNAT) activity *in vivo* and *in vitro* [6].

MATERIALS AND METHODS

Pineal glands were obtained from male rats, of the Hebrew University's "Sabra" strain, weighing 180–200 g each, kept in alternating light and darkness (lights on at 6:00 a.m. and off at 6:00 p.m. daily). The animals were decapitated between 4 and 5 hr after the lights were turned on, and their pineals were removed immediately and placed in the culture media and incubated overnight for a total of 21 hr.

The pineals were incubated in Wassermann tubes with 0.4 ml of the nutrient medium of Shein *et al.* [7] containing 330 nCi *d,l*-[methylene-^{14}C]tryptophan (Amersham International, Amersham, UK) in a 10^{-4} M tryptophan solution and the respective test substances, CPZ, HAL, DIP or norepinephrine (NE) HCl, in a 10 μl aqueous solution to produce a final concentration of 1×10^{-5} M. Each tube, containing one pineal that was immersed and floated freely in the medium, was closed tightly with a rubber stopper and incubated at 37° in a rotating roller wheel for 21 hr according to Wurtman *et al.* [8]. Control tubes were incubated with pineals and [^{14}C]tryptophan without added test substances. Blanks were taken from tubes incubated with [^{14}C]tryptophan and added test substances without pineal glands.

On termination of incubation, the tubes were stored at −15° until assay. For the quantitative assay, 100-μl aliquots of the medium were combined with 20 μl of indoleamine solution containing 5-HT, NAS, MT, 5-hydroxytryptophol (5-HTOH) and 5-hydroxyindoleacetic acid (5-HIAA), and 50 μl of this mixture was applied to a precoated plastic sheet with TDC (Polygram Sil G/UV$_{254}$/10 × 10 cm Macheray-Nagel Co., Düren, West Germany) under a continuous stream of nitrogen. Chromatographs were developed in darkness in a solvent system (chloroform–methanol–acetic acid, 90:10:1) repeated twice in the same dimension. The sheets were rapidly dried, and a strip containing the initial spot was cut off from the lower part of the sheet.

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Table 1. Effects of chlorpromazine, haloperidol and desipramine on basal and norepinephrine-induced metabolism of [^{14}C]tryptophan in a rat pineal culture medium*

Drugs in media at M^{-5} concn	[^{14}C]Indoleamines produced in medium/pineal (dpm ± S.E.M.)				
	NAS	MT	5-HTOH	5-HIAA	5-HT
Control	224.2 ± 16.9 (28)	228.8 ± 19.1 (29)	1238.0 ± 91.0 (23)	1604.2 ± 78.1 (26)	3591 ± 157 (31)
Control + NE	941.8 ± 128.8† (17)	974.9 ± 75.0† (17)	899.1 ± 39.3‡ (21)	1179.1 ± 63.2† (23)	3126 ± 126§ (29)
CPZ	442.5 ± 4.32† (26)	610.4 ± 44.0† (26)	859.1 ± 59.3‡ (20)	1262.4 ± 90.7‖ (21)	3871 ± 150 (28)
CPZ + NE	1559.6 ± 141.5¶ (16)	1435.4 ± 98.8** (16)	738.7 ± 41.8†† (22)	917.7 ± 64.4‡‡ (21)	2975 ± 112 (32)
HAL	172.6 ± 13.8 (26)	319.1 ± 19.2‖ (23)	1212.7 ± 47.9 (25)	1370.2 ± 64.4§§ (25)	4690 ± 237† (18)
HAL + NE	762.6 ± 37.0 (16)	1130.6 ± 64.5‡‡ (23)	1116.3 ± 44.1** (26)	1179.0 ± 48.5 (25)	4140 ± 165** (19)
DIP	372.0 ± 28.4† (29)	491.6 ± 31.8† (29)	824.0 ± 55.8† (20)	1332.3 ± 67.0‖ (19)	2789 ± 172† (30)
DIP + NE	1349.9 ± 126‡‡ (16)	1305.0 ± 105†† (16)	673.0 ± 41.3** (20)	888.9 ± 40.4¶ (20)	2657 ± 137‡‡ (32)

* Abbreviations: CPZ, chlorpromazine; HAL, haloperidol; DIP, desipramine; NE norepinephrine; NAS, *N*-acetylserotonin; MT, melatonin; 5-HTOH, 5-hydroxytryptophol; 5-HIAA, 5-hydroxyindole acetic acid; and 5-HT, serotonin. The number in parentheses equal the number of pineals.

† $P < 0.001$, ‡$P < 0.005$, §$p \leq 0.05$, and ‖$P < 0.01$, compared to control.

¶$P < 0.005$, **$P < 0.001$, ††$P < 0.01$, and ‡‡$P \leq 0.05$, compared to control + NE.

§§$P < 0.02$, compared to control.

This strip was run in the second direction in a system composed of isopropanol–methylacetate–ammonium hydroxyde 25% (45:35:20). This additional procedure was necessary in order to separate the 5-HT from tryptophan and 5-hydroxytryptophan. The rest of the sheet, containing all other indoleamines, was placed in ethylacetate for development in darkness in the second direction. The separated metabolites of [^{14}C]tryptophan were marked under a u.v. lanp, cut out from the sheet and placed in small counting vials. Then 4 ml of scintillation fluid was added, and the sample was counted by standard liquid scintillation techniques.

RESULTS

Substantially more [^{14}C]Mt was recovered from the cultures incubated with either the neuroleptics, CPZ and HAL, or the antidepressant, DIP, than from the media not containing these test substances (Table 1). With CPZ and DIP also, the level of the [^{14}C]MT precursor [^{14}C]NAS was elevated. The amount of [^{14}C]HIAA, the final metabolite of [^{14}C]5-HT, was found to be significantly decreased with all three drugs tested. With CPZ and DIP the concentration of the [^{14}C]5-HT intermediate metabolite, [^{14}C]5-HTOH, was also reduced. The levels of [^{14}C]5-HT that were produced when [^{14}C]tryptophan was added to the medium were found to be increased, although not significantly so, in the presence of CPZ, significantly elevated with HAL, but reduced with DIP. The addition of NE to the culture medium brought about more than a 4-fold increase in [^{14}C]NAS and [^{14}C]MT at the time measured, and a decrease in [^{14}C]5-HTOH, [^{14}C]5-HIAA and [^{14}C]5-HT. Combination of NE with either CPZ or DIP, but not with HAL, brought about significantly higher levels of [^{14}C]NAS and lower levels of [^{14}C]5-HTOH and [^{14}C]5-HIAA; [^{14}C]MT was elevated with each of the three drugs tested in combination with NE. The [^{14}C]5-HT values, found to be depressed in the presence of NE relative to the controls, were even further decreased with DIP, increased with HAL, and unaffected by CPZ.

DISCUSSION

CPZ of the phenothiazine group, and HAL, a butyrophenone compound, both of which are effective antipsychotic neuroleptic drugs, were found to bring about elevated levels of MT (in the case of CPZ and, also, of the MT precursor NAS) in a rat pineal culture medium containing [^{14}C]tryptophan. This would indicate stimulation of either or both enzymes involved in the synthesis of MT, HIOMT and SNAT. A simultaneous decrease in 5-HT oxidative deamination, as evidenced by diminished concentrations of its metabolites 5-HTOH and/or 5-HIAA, was recorded.

Haloperidol was the only compound shown to elevate significantly 5-HT; DIP depressed 5-HT levels. The high level of 5-HT in the presence of HAL suggests that, in addition to the already stated effect of decreased oxidative deamination evidently due to inhibition of the monoamine oxidase enzyme, a possibility exists of accelerated 5-HT synthesis due to 5-tryptophan hydroxylase stimulation, although a potentiated tryptophan uptake by the cultured pineal gland cannot be excluded. Such an assumption would be justified on the basis of the finding that 5-HT, in

spite of its potentiated conversion to MT, accumulated in the culture medium as compared to the controls or to the antidepressant drug DIP. Thus, it may be suggested that the presence of neuroleptics in a pineal culture medium could bring about, in addition to accelerated conversion of 5-HT to MT, two basic changes in the reactions involved in tryptophan metabolism: 5-tryptophan hydroxylase stimulation and/or monoamine oxidase inhibition.

Potentiated MT synthesis and SNAT activity were also found for the tricyclic antidepressant DIP. However, in its presence 5-HT concentration in the pineal culture medium was decreased, indicating reduced synthesis from tryptophan, evidently by blockage of 5-tryptophan hydroxylase activity. This effect is the opposite of that recorded with the neuroleptic CNS-depressant HAL.

Although MT has been implicated in sleep and sedation [9], both types of psychotropic drugs—the CNS-depressant neuroleptics and the tricyclic antidepressants—potentiate MT production and depress 5-HT oxidative deamination. Our findings indicate that the difference between the actions of the two types of compounds lies in their abilities to synthesize 5-HT *de novo*. While the rate of synthesis was potentiated by the neuroleptic, it was blocked by the antidepressant. But, in spite of the increased availability of 5-HT, here too there was a persistent diminished oxidative deamination.

Our findings of increased NAS and MT production from tryptophan in pineal culture medium differ from those obtained in direct *in vitro* studies were neuroleptics inhibited HIOMT activity [4] and indicate that the increased MT level brought about by neuroleptics, found *in vivo*, may not necessarily be due to a reduced rate of MT metabolism in the body [1–3].

How, then, do the drugs tested affect NE-induced changes of tryptophan metabolism in a pineal culture medium? NE, released *in vivo* in the pineal, stimulats β-adrenergic receptor-coupled adenylate cyclase, and the cyclic AMP produced leads to increased protein synthesis, increased SNAT activity, and increased MT synthesis.

In our pineal culture medium containing [^{14}C]tryptophan, NE brought about elevated synthesis of NAS and MT and reduced oxidative deamination (but a lower level) of 5-HT. The two neuroleptics, CPZ and HAL, affected these NE-induced responses differently. CPZ acted in the same manner as in the basal tryptophan metabolism, i.e. by activating the MT produciton, reducing further 5-HT catabolism and bringing about no change in 5-HT levels. CPZ, although having the same effect as NE on [^{14}C]tryptophan metabolism, may not necessarily act primarily by increasing NE release from the noradrenergic nerve endings, especially since it continues to exert an additional effect in the presence of NE. Our system was a combination of an innervated pineal gland with active noradrenergic nerve endings, at first, and, later, an essentially denervated organ. With HAL we found a marginal enhancement of the NE-induced MT synthesis only; other variables were either not affected or were shifted in the opposite direction, cancelling out the NE effect, since the 5-HT decline was completely averted.

The antidepressant DIP, as in the case of the basal effects, potentiated further NE-induced elevation of MT synthesis and reduced 5-HT oxidative deamination, similar to CPZ. The NE-induced effect on 5-HT production, however, was affected by DIP differently than by the two neuroleptics; it significantly depressed further the already inhibited synthesis, contrary to HAL, which eliminated the NE-induced inhibition, and differently from CPZ, which had no effect on the NE-induced depression of 5-HT.

Thus, as previously noted with its basal effects, the antidepressant, DIP, differed from the CNS-depressant neuroleptic, not in MT synthesis and 5-HT catabolism, but in its effect on 5-HT production. In conclusion, the same pattern of reactions of increased formation of MT with decreased conversion of 5-HT to its metabolites, 5-HTOH and/or 5-HIAA, was shared by various groups of psychotropic drugs with entirely different mechanisms of pharmacologic action, i.e. CNS-depressant dopamine-blocking neuroleptics, the tricyclic antidepressants, which are NE and 5-HT reuptake inhibitors, and, as reported in the literature, the psychotomimetic central stimulants [10, 11].

The difference recorded by us, between the depressant and antidepressant groups of drugs, was in the rate of tryptophan conversion to 5-HT, which may be a clue to the dissimilitude of the pharmacological effects generated by long-term treatment with these two types of psychotropic agents.

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