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# Effects of 6-Methoxy-1,2,3,4-tetrahydro-β-carboline and Yohimbine on Hypothalamic Monoamine Status and Pituitary Hormone Release in the Rat

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*Abstract*

Shortly after administration of 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeOTHBC) and yohimbine to normal or hypothyroid rats [the latter exhibiting chronically elevated levels of serotonin (5-HT) neuronal activity in the hypothalamus] there was a highly significant increase in hypothalamic noradrenaline (NA) activity and in ACTH release concomittant with a reduction in hypothalamic 5-HT activity ($P<0\cdot01$) and in growth hormone (GH) ($P<0\cdot01$) and in thyroid stimulating hormone (TSH) ($P<0\cdot01$) release from the pituitary. Both compounds caused an increase in hypothalamic dopamine (DA) metabolism and in pituitary prolactin (PRL) release in normal rats ($P<0\cdot01$) but only yohimbine exerted this action in hypothyroid rats. Lower doses of 6-MeOTHBC exerted a relatively specific effect in hypothyroid rats, reducing ($P<0\cdot01$) 5-HT neuronal activity in parallel with pituitary TSH secretion ($P<0\cdot05$). While gross effects of 6-MeOTHBC and yohimbine were similar with respect to their effects on NA and 5-HT status in the hypothalamus, there were quantitative differences. 6-MeOTHBC always caused a greater decrease in 5-HT turnover and a lesser increase in NA turnover than did yohimbine.

On the basis of these studies we suggest that the effect of tetrahydro-β-carboline-related alkaloids on pituitary hormone release may be due to their influence on hypothalamic monoamine status and the subsequent alteration of the hypothalamic–pituitary control system.

## Introduction

In 1934 Hahn and Ludewig demonstrated that, under physiological conditions, acetaldehyde will condense with tryptamine. The product of this Pictet–Spengler condensation is 1-methyl-1,2,3,4-tetrahydro-β-carboline (THBC). Substituted THBC's may be formed by condensation of an aldehyde with substituted tryptamines such as serotonin (5-hydroxytryptamine, 5-HT). Interest in these compounds and the related tetrahydroisoquinoline alkaloids derived from aldehyde–catecholamine condensation has centred largely on their possible endogenous production following ethanol consumption (Deitrich and Erwin 1980). It is notable that members of both the THBC and tetrahydroisoquinoline class of alkaloids have recently been reported to occur in foodstuffs such as fruit and fermented beverages (Duncan and Smythe 1982; Beck *et al.* 1983).

Evidence has been presented suggesting that alkaloids of the THBC class may occur endogenously and are able to effect a wide range of neurochemical, neuroendocrine and behavioural actions (Buckholtz 1980*a*; Deitrich and Erwin 1980; Barker *et al.* 1981; Rommelspacher *et al.* 1982). In particular, 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeOTHBC) has been well studied and has been reported

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to be present endogenously in rat brain and adrenal tissue (Barker *et al.* 1981) and has been shown to cause increased secretion of corticosterone in mice (Meyer and Buckholtz 1976). Administration of 6-MeOTHBC to mice results in elevated 5-HT concentrations and reduced concentrations of the major metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) in the brain. These changes were proposed (Buckholtz 1980*b*) to be primarily due to inhibition by 6-MeOTHBC of neuronal 5-HT uptake. Regardless of its precise mode of action, the ability of 6-MeOTHBC to markedly reduce the ratio of 5-HIAA/5-HT in mouse brain strongly indicates that this compound reduces central 5-HT neuronal activity (see Smythe *et al.* 1982*a*, 1982*b*). As a consequence 6-MeOTHBC should inhibit the secretion of pituitary growth hormone (GH) and thyroid stimulating hormone (TSH) which are both under stimulatory control by hypothalamic 5-HT neuronal activity (Smythe *et al.* 1982*a*, 1982*b*). Furthermore, the stimulatory effect of 6-MeOTHBC on corticosterone release in mice indicates that this compound also increases central noradrenaline (NA) neuronal activity which we have shown to be a specific stimulus to pituitary adrenocorticotrophin (ACTH) release (Smythe *et al.* 1983). The reported effects of 6-MeOTHBC bear similarity to some actions we have shown to be exerted by another alkaloid, yohimbine, which possesses the THBC nucleus. Yohimbine is an α-adrenergic antagonist which is a powerful stimulator of hypothalamic NA neuronal activity and pituitary ACTH release but which simultaneously causes a reduction in the hypothalamic ratio of 5-HIAA/5-HT in the rat (Smythe *et al.* 1983).

The concept that the neuronal activities of brain monoamines may be assessed from the ratio of the neuronal metabolite concentration to the concentration of the parent monoamine (Hery *et al.* 1972; Mena *et al.* 1976; Heffner *et al.* 1980) is well supported by theory and practice (Smythe *et al.* 1983). Computerized gas chromatography–mass spectrometry using deuterated internal standards provides a method of analysing brain monoamine and metabolite concentrations with high precision and specificity (Smythe *et al.* 1982*a*, 1983). In the present investigation this technique was used to obtain the concentrations of hypothalamic monoamines and their metabolites for the assessment of monoamine neuronal activity by the metabolic ratio method. In order to elucidate the neuroendocrine actions of 6-MeOTHBC and yohimbine we have examined their effects on hypothalamic monoamine (DA, NA and 5-HT) neuronal activity and concurrent pituitary hormone release. Two rat models were used in which to compare the effects of the two alkaloids; the normal 'gentled' rat (subjected to daily handling and mock injections for 10 days) and the hypothyroid rat which exhibits chronically elevated hypothalamic 5-HT neuronal activity in association with elevated TSH secretion (Smythe *et al.* 1982*b*).

## Materials and Methods

The GC/MS assays for DA, NA, 5-HT, homovanillic acid (HVA), 3,4-dihydroxyphenylethyleneglycol (DHPG) and 5-hydroxyindoleacetic acid (5-HIAA) were carried out using a Hewlett-Packard 5993A GC/MS data system (Hewlett Packard Australia Pty. Ltd., North Ryde, N.S.W., Australia) with deuterated internal standards, extraction and derivatization procedures being identical to those previously described (Smythe *et al.* 1982*a*). The between-assay coefficient of variation for the various compounds assayed ranged from 2·5 to 7·0%. Sensitivity was less than 200 fmol for each compound. All results are expressed as picomoles per milligram of tissue wet weight.

### *Reference Compounds and Drugs*

The results reported in this study are uncorrected and refer to the respective free bases, acids and alcohols. Each of the following reference compounds was dried *in vacuo* before use: DA

hydrochloride (Calbiochem-Behring, Carlingford, Australia), L-NA bitartrate (Calbiochem-Behring), 5-HT–creatinine sulfate complex (Sigma Chemical Co.), HVA (Sigma Chemical Co.), DHPG (Calbiochem-Behring) and 5-HIAA (Calbiochem-Behring).

Yohimbine hydrochloride and 6-*N*-propylthiouracil (PTU) were obtained from Sigma Chemical Co. 6-MeOTHBC was chemically synthesized by one of us (M.W.D.) by cyclization of 5-methoxytryptamine with glyoxalic acid using a modification of the method of Ho *et al.* (1968).

*Radioimmunoassay*

The corticosterone concentration in rat serum samples was assayed by a modification of the method of Carr *et al.* (1977). The sensitivity of the assay, defined as the smallest amount distinguishable from zero with 95% confidence, was 5 nmol/l. The accuracy was assessed by adding various concentrations of corticosterone standard to a number of samples prior to assay; the average percentage recovery of standard was 97±4 (s.d.) ($n$ = 10). The precision of the assay as estimated by the intra-assay coefficient of variation was 5·1% at 92 nmol/l, 4·1% at 450 nmol/l and 4·5% at 920 nmol/l ($n$ = 10, 12, 10 respectively). The interassay coefficients of variation were 12·0, 9·3, and 11·9% ($n$ = 11, 14, 11) respectively. A number of rat serum samples were assayed in various dilutions and the measured corticosterone concentration was found to be independent of the volume of serum assayed. Serum ACTH was estimated by radioimmunoassay using materials supplied by Immuno Nuclear Corporation (Stillwater, Minnesota). The limit of sensitivity for the assay was 50 pg/ml and the intra- and interassay coefficients of variation were 15% and 30% (maximum) respectively. Serum and rat GH (rGH), rat PRL (rPRL) and rat TSH (rTSH) were assayed in double antibody radioimmunoassay using material supplied by Dr A. Parlow (N.I.A.M.D.D., Bethesda, MD.). Data are expressed in terms of rGH-RP-1, rPRL-RP-2 and rTSH-RP-1. The interassay coefficients of variation were less than 9%. All samples were assayed in duplicate.

*Statistics*

Statistical analyses were carried out using Student's $t$-test and the multiple comparison test of Scheffé (see Dunnett 1970).

*Animal Studies*

Outbred male rats of the Wistar strain were used throughout these studies. The animals were fed *ad libitum* and subjected to 12 h dark–12 h light cycles. All experiments were concluded between 0930–1100 hours. All animals were killed by decapitation at which time trunk blood was collected. Brains were rapidly removed and the medial basal hypothalamus (MBH) was extracted using a fine glass punch (3·00 mm i.d.) with a Teflon plunger; the centre of the punch was positioned on the saggital midline and coordinate A 4·0 (Konig and Klippel 1963). The basal portion of this section (approx. 2·0 mm) was taken as the MBH. The mean wet weight of the MBH samples for all the studies reported here was 18·0±1·8 (s.d.) mg. MBH samples were mechanically homogenized (Ultra-Turrax) in 1 ml of a solution of 5 M formic acid in n-butanol (1:4 v/v) which contained calibrated amounts of deuterated standards for the monoamines and metabolites (see Smythe *et al.* 1982*a*).

*Study 1: Effects of 6-MeOTHBC and Yohimbine Administration to Gentled Normal Rats*

Eighteen normal male rats 60 days old and weighing 250–270 g were divided into three equal groups and were subjected to 'gentling' by daily handling and mock injections for the 10 days preceding the experiment at which time the control group was administered 1 ml saline (i.p.). The other two groups were administered 6-MeOTHBC (50 mg/kg i.p.) or yohimbine hydrochloride (8 mg/kg i.p.) dissolved in 1 ml saline. The animals were killed 30 min after injection.

*Study 2: Effects of 6-MeOTHBC and Yohimbine Administration to Hypothyroid Rats*

Twenty-three male rats 48 days old were maintained on PTU (0·05% w/v) in their drinking water for 13 days preceding the experiment. The animals were divided into four groups (A–D). Group A (controls, $n$ = 5) was administered saline (1 ml, i.p.); group B ($n$ = 5) was administered 6-MeOTHBC (5 mg/kg i.p.); group C ($n$ = 6) was administered 6-MeOTHBC (50 mg/kg i.p.); group D ($n$ = 6) was administered yohimbine hydrochloride (8 mg/kg i.p.). All drugs were dissolved in saline and the animals were killed 30 min after injection.

## Results

*Study 1*

The administration of either 6-MeOTHBC or yohimbine resulted in marked effects on hypothalamic DA, NA and 5-HT status as shown in Table 1. 6-MeOTHBC caused increases in both HVA and DA concentrations ($P<0\cdot01$) in the hypothalamus but no significant change in the ratio of HVA/DA. On the other hand yohimbine caused an increase in HVA ($P<0\cdot01$) but no change in DA concentration with the net result that the ratio HVA/DA was increased ($P<0\cdot01$). Both MeOTHBC and yohimbine exerted similar effects on NA and 5-HT concentrations, falls in NA and 5-HIAA concentrations ($P<0\cdot01$), elevation of the ratio DHPG/NA ($P<0\cdot01$) and a marked fall in the ratio 5-HIAA/5-HT ($P<0\cdot01$) being found. Also shown in Table 1 are the comparable and highly significant effects of these two compounds on pituitary hormone and corticosterone secretion. The serum levels of ACTH, corticosterone and PRL were markedly increased while GH secretion was markedly suppressed with both 6-MeOTHBC and yohimbine.

**Table 1. Hypothalamic concentrations and metabolism of DA, NA, and 5-HT and serum hormone levels in normal male rats 30 min after treatment with saline (controls), 6-MeOTHBC (50 mg/kg, i.p.) or yohimbine-HCl (8 mg/kg, i.p.)**

Means ±s.e.m. are shown. Superscripts: a, $P<0\cdot01$ *v.* controls; b, not significantly different from controls; $P<0\cdot05$ *v.* controls; d, $P<0\cdot05$ *v.* 6-MeOTHBC group

| | Controls ($n = 6$) | 6-MeOTHBC ($n = 6$) | Yohimbine ($n = 6$) |
|---|---|---|---|
| HVA (pmol/mg) | 0·65±0·045 | 1·04±0·075[a] | 0·90±0·02[a] |
| DA (pmol/mg) | 4·58±0·27 | 6·22±0·30[a] | 4·73±0·24[b] |
| Ratio HVA/DA | 0·146±0·016 | 0·169±0·016[b] | 0·196±0·015[c] |
| DHPG | 1·07±0·07 | 1·54±0·09[a] | 1·55±0·05[a] |
| NA | 16·6±1·45 | 11·80±0·47[a] | 9·45±0·28[a,d] |
| Ratio DHPG/NA | 0·065±0·003 | 0·131±0·008[a] | 0·163±0·006[a,d] |
| 5-HIAA (pmol/mg) | 3·98±0·25 | 1·96±0·10[a] | 2·67±0·25[a] |
| 5-HT (pmol/mg) | 6·42±0·25 | 9·14±0·58[a] | 8·40±0·52[a] |
| Ratio 5-HIAA/5-HT | 0·64±0·04 | 0·22±0·014[a] | 0·32±0·02[a,d] |
| Serum GH (ng/ml) | 404±15·2 | 13·8±1·1[a] | 13·0±2·0[a] |
| Serum PRL (ng/ml) | 34±14 | 93±13[a] | 117±20[a] |
| Serum ACTH (pg/ml) | 99±15·4 | 580±26[a] | 566±52[a] |
| Serum corticosterone (mmol/l) | 272±15·3 | 1283±34[a] | 1150±34[a] |

*Study 2*

The hypothyroid rats used in this study showed typically high hypothalamic ratios of 5-HIAA/5-HT and HVA/DA (Smythe *et al.* 1982*b*). The low dose (5 mg/kg) administration of 6-MeOTHBC caused no change to either DA or NA status in the hypothalamus but resulted in a reduction of hypothalamic 5-HIAA concentration ($P<0\cdot01$) and an increase in 5-HT concentration ($P<0\cdot05$) (see Table 2). As shown in Table 2 low dose 6-MeOTHBC significantly altered the secretion of TSH only. The administration of high dose 6-MeOTHBC and yohimbine resulted in more marked changes in hypothalamic monoamine and hormone status

as shown in Table 2. 6-MeOTHBC caused a decrease in the hypothalamic ratio of HVA/DA and also in the ratio of 5-HIAA/5-HT (both $P<0{\cdot}01$) while the ratio of DHPG/NA was increased ($P<0{\cdot}01$). With the exception of HVA, which was not significantly altered after 6-MeOTHBC, the other monoamine parameters were highly significantly different. While, in this case, there was no significant change in PRL secretion both GH and TSH secretion were reduced ($P<0{\cdot}01$) and corticosterone

**Table 2. Hypothalamic concentrations and metabolism of DA, NA and 5-HT and serum hormone levels in hypothyroid (PTU-treated) male rats 30 min after saline (group A), low (group B) and high (group C) doses of 6-MeOTHBC and yohimbine-HCl (group D)**

Means ±s.e.m. are shown. Superscripts: a, not significantly different from group A; b, $P<0{\cdot}05$ *v.* group A; c, $P<0{\cdot}0125$ *v.* group A; d, $P<0{\cdot}01$ *v.* group A; e, $P<0{\cdot}05$ *v.* Group C

| | Group A: Controls (saline 1 ml i.p.) ($n$ = 6) | Group B: 6-MeOTHBC (5 mg/kg i.p.) ($n$ = 5) | Group C: 6-MeOTHBC (50 mg/kg i.p.) ($n$ = 6) | Group D: Yohimbine (8 mg/kg i.p.) ($n$ = 6) |
|---|---|---|---|---|
| HVA | 1·50±0·07 | 1·53±0·07[a] | 1·56±0·10[a] | 1·88±0·14[b] |
| DA | 2·50±0·095 | 2·49±0·17[a] | 3·18±0·21[c] | 3·09±0·15[d] |
| Ratio HVA/DA | 1·02±0·06 | 0·99±0·06[a] | 0·79±0·04[d] | 1·39±0·05[d] |
| DHPG | 1·24±0·08 | 1·26±0·07[a] | 1·82±0·07[d] | 2·79±0·17[d] |
| NA | 11·80±0·42 | 11·57±0·69[a] | 10·62±0·40[b] | 7·45±0·27[d] |
| Ratio DHPG/NA | 0·105±0·006 | 0·11±0·003[a] | 0·172±0·005[d] | 0·375±0·019[d,e] |
| 5-HIAA | 4·59±0·14 | 3·49±0·13[d] | 2·78±13[d] | 3·27±0·24[d] |
| 5-HT | 4·26±0·19 | 4·90±0·15[b] | 5·20±0·23[c] | 5·21±0·20[d] |
| Ratio 5-HIAA/5-HT | 1·08±0·03 | 0·72±0·03[d] | 0·54±0·02[d] | 0·63±0·03[d,e] |
| Serum GH (ng/ml) | 66±14 | 45±9[a] | 33±8[b] | 25±6[d] |
| Serum PRL (ng/ml) | 50±13 | 73±15[a] | 61±8[a] | 85±8[b] |
| Serum TSH (μU/ml) | 2409±212 | 1716±144[b] | 1492±97[d] | 1538±91[d] |
| Serum corticosterone (nmol/l) | 164±9 | 240±45[a] | 880±20[d] | 1090±44[d,e] |

secretion was increased ($P<0{\cdot}01$). The gross effects of yohimbine administration to these hypothyroid rats were similar to its effects in normal rats seen in study 1 above. Yohimbine caused highly significant increases in the hypothalamic ratios of HVA/DA and DHPG/NA and a highly significant decrease in the ratio of 5-HIAA/5-HT. The secretion of PRL and corticosterone was increased after yohimbine while the secretion of both GH and TSH was reduced.

## Discussion

The data from this investigation demonstrate that the substituted THBC alkaloids 6-MeOTHBC and yohimbine exert important actions on central monoamine neuronal activity and pituitary hormone release. The effects of 6-MeOTHBC on 5-HT and 5-HIAA concentrations in the hypothalamus are consistent with the effects of the same dose (50 mg/kg) of this compound on whole mouse brain 5-HT and 5-HIAA observed by Buckholtz (1980*b*). It has been previously reported that 6-MeOTHBC has no effect on whole mouse brain NA concentrations (McIsaac *et al.* 1972; Buckholtz 1980*a*). In the present study the higher dose of 6-MeOTHBC caused

significant falls in hypothalamic NA concentration with the concurrent increase in NA neuronal activity. Little previous data is available on the effects of 6-MeOTHBC on pituitary hormone secretion. The inhibitory action of this alkaloid on GH and TSH secretion in the present study would have been predicted from its previously reported effect on brain 5-HT status (Buckholtz 1980*b*) taken with our own recent data relating to the relationship between hypothalamic 5-HT neuronal activity and rGH and rTSH release. (Smythe *et al.* 1982*a*, 1982*b*). Similarly the effect of the higher dose 6-MeOTHBC in stimulating ACTH and corticosterone secretion was consistent with our previous studies on the relationship between ACTH stimulation and high NA neuronal activity (Smythe *et al.* 1983). The lower dose of 6-MeOTHBC used in the study with hypothyroid rats exerted a less marked but relatively specific suppressive effect on 5-HT neuronal activity. There was also concurrent reduction in the high levels of circulating TSH, but no significant reduction of GH secretion was observed. This lack of effect on GH following suppression of hypothalamic 5-HT activity in hypothyroid rats has been previously demonstrated by us with other drugs such as cyproheptadine and *p*-chlorophenylalanine and is possibly due in part to gross depletion of pituitary GH stores (Smythe *et al.* 1982*b*). Presumably, the ability of high dose 6-MeOTHBC and also that of yohimbine to inhibit further GH release in the hypothyroid rat model is due to a greater suppression of hypothalamic 5-HT activity which occurs with these alkaloids in association with increased NA activity.

The brain hormone relationships reported here for normal and hypothyroid rats following yohimbine administration are the same as we have previously shown (Smythe *et al.* 1983) with respect to hypothalamic NA neuronal activity and pituitary ACTH release and are qualitatively similar to those for 6-MeOTHBC (higher dose) and suggest that 6-MeOTHBC possesses some $\alpha_2$-noradrenergic receptor antagonist properties. Quantitively, however, there are differences in the relative effects of the two alkaloids on hypothalamic monoamine status. Yohimbine exerted significantly more powerful stimulation of NA neuronal activity than did 6-MeOTHBC and this effect was greatest in the hypothyroid rats where serum corticosterone levels were significantly higher in the yohimbine-treated group of animals than in that treated with 6-MeOTHBC. On the other hand the inhibition of hypothalamic 5-HT activity was greater in the case of 6-MeOTHBC. There were also significantly different effects of the two alkaloids with respect to hypothalamic DA status. The data with respect to 6-MeOTHBC suggest that the higher dose of this compound exerted DA receptor antagonism and blockade of DA action. The responses of hypothalamic DA and HVA and the marked rise in serum PRL following the administration of 6-MeOTHBC in normal rats were typical of receptor blockade-induced compensatory increase in synthesis; however, in hypothyroid rats, in which DA synthesis rate is already high, the receptor blockade resulted in a fall in DA turnover and neural activity. The increases in the hypothalamic ratio of HVA/DA seen following yohimbine administration are not entirely consistent with DA receptor antagonism and may reflect a high degree of DA utilization in its role as NA precursor in response to the increased demand for NA synthesis in this case of $\alpha_2$-adrenoceptor blockade. However, the data, taken with the increased prolactin secretion seen following yohimbine administration, would be consistent with increased utilization of DA in NA synthesis occurring together with blockade of the prolactin-inhibitory function of DA.

These data indicate that the type and degree of substitution on the THBC nucleus can result in significant differences in modulating monoamine activities in the central nervous system and that the specific properties of each putative biogenic THBC needs to be established in detail. However, general similarities of effects of 6-MeOTHBC and yohimbine shown in this study give credence to hypotheses that alkaloids of this type, whether administered exogenously (perhaps in foodstuffs) or formed endogenously, may have important neuroendocrine effects via their alteration of hypothalamic monoamine neuronal activity and consequent disturbance of hypothalamic–pituitary control mechanisms.

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