Serum Prolactin and Brain and Pituitary Monoamine Responses to Chronic Monoamine Oxidase Inhibition in the Rat

G. A. Smythe and J. F. Brandstater
Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney 2010.

Abstract
The acute administration of the monoamine oxidase inhibitor iproniazid to rats causes a highly significant suppression of serum prolactin levels at 2 h. At the same time there is a significant rise in the hypothalamic–median eminence concentrations of the biogenic monoamines dopamine, noradrenaline and serotonin. When iproniazid is administered daily to rats for 4 days and the animals are examined on the fifth day brain noradrenaline and serotonin levels are elevated similarly to those seen after acute administration but dopamine concentration is near normal while serum prolactin is significantly elevated. This study thus demonstrates that a quite specific and unexpected change occurs in the regulation of hypothalamic–median eminence dopamine when iproniazid is administered chronically and provides an explanation of previous observations in human subjects where raised serum prolactin levels are observed after chronic therapy with monoamine oxidase inhibitors.

Extra key-words: 5-hydroxy-L-tryptophan; biogenic monoamines; L-dopa.

Introduction
The acute administration of monoamine oxidase inhibitors (MAOI) to rats causes a significant reduction in serum prolactin levels (Lu and Meites 1971)—an effect presumed to be due to increased concentrations of hypothalamic dopamine (DA) and consequent increased prolactin inhibiting factor (PIF) activity (Lu and Meites 1971). The more recent observation that plasma prolactin is elevated in depressed patients treated with MAOI (Slater et al. 1977) raised the questions whether there is a species difference in the prolactin responses to MAOI or whether there are differences in the acute versus chronic effects of MAOI.

This study was aimed at resolving these questions by examining the chronic effects on rat prolactin secretion of MAOI using iproniazid and to determine the status of hypothalamic–median eminence and pituitary biogenic monoamines in this situation. The possibility of a shift in balance between dopaminergic inhibition and possible serotoninergic stimulation of prolactin secretion following chronic MAOI which was raised by Slater et al. (1977) was also studied by including an examination of the effects of the DA precursor L-dopa and the serotonin (5-HT) precursor 5-hydroxy-L-tryptophan (5-HTP) on prolactin secretion in rats following chronic MAOI.
Materials and Methods

Experimental Animals

Normal adult male rats of the Wistar strain weighing approximately 200 g were used in this study. The animals were housed in a room in which the ambient temperature varied from 18 to 22°C with a lighting schedule which provided 12-h dark–light cycles. Food and water were available ad libitum.

Drugs

The drugs employed in this investigation were the following: iproniazid (Marsilid, Roche Products Pty. Ltd., Sydney); L-dopa (3,4-dihydroxy-L-phenylalanine, Merck, Darmstadt); and 5-HTP (Sigma Chemical Co., St. Louis).

Experimental Procedures

In all experiments drugs were injected intraperitoneally and, unless stated otherwise, on the day of slaughter animals were killed by decapitation 30 min following the drug injection. Anterior pituitary and hypothalamic and median eminence sections of the brains were rapidly dissected and homogenized in n-butanol–formic acid. These samples were assayed for DA, 5-HT and noradrenaline (NA) using the quantitative mass spectrometry (selected ion monitoring) procedure previously described (Smythe et al. 1979). Results are expressed in terms of the wet weight of tissue.

Blood serum was collected and stored at –20°C until assayed for prolactin by radioimmunoassay using reagents supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Hormone Program, Bethesda, Md. The lower limit of sensitivity of the prolactin radioimmunoassay was 100 pg (two standard deviations (s.d.) from total label bound in the absence of added prolactin standard) and the upper limit was 15 ng (two s.d. below total label bound in presence of highest prolactin standard). Serum samples (100 μl) were assayed in duplicate and in this study none had prolactin concentrations falling outside the sensitivity limits of the assay. The interassay coefficient of variation was 9% or less for a range of control sera.

Dose of Drugs

The dose of the MAOI iproniazid used in this investigation was 50 mg kg⁻¹ which represents approximately one-quarter of the dose of this drug used by Lu and Meites (1971). In preliminary experiments we established that the dose chosen was more than adequate to achieve significant monoamine oxidase inhibition in vivo. Both L-dopa and 5-HTP were administered in a dose of 10 mg kg⁻¹. At this dose level L-dopa causes a significant suppression of prolactin secretion in normal rats while this dose of 5-HTP has no effect on prolactin in normal rats (Smythe and Lazarus 1973). All drugs were administered in physiological saline (0·5 ml).

Study 1

In order to establish the effect of acute MAOI on hypothalamic biogenic monoamines and prolactin secretion, one group of five rats was injected with physiological saline (controls) and another group of five rats was administered iproniazid. Both groups were killed 2 h post-injection. For the purposes of this study the brain monoamines were measured only in the hypothalamus.

Study 2

To determine the effects of chronic MAOI one group of five rats was injected daily for 4 days with saline (controls) and a further 15 rats were injected daily for 4 days with iproniazid. On the fifth day of the study (day of slaughter) the controls were given a further injection of saline. The animals which had been treated with iproniazid were subdivided into three groups of five. One of these groups was administered saline alone, one received L-dopa and the others 5-HTP.

Statistics

All data were evaluated statistically using Student's t-test and results are expressed as the mean ± s.e.m.
Results

The hypothalamic concentration of DA, NA and 5-HT were all significantly elevated following the acute, single dose of iproniazid (see Table 2). At the same time there was a highly significant fall \((P<0.0005)\) in serum prolactin. The effect of chronic MAOI with iproniazid on serum prolactin was quite different and is shown in Table 1. In this case prolactin secretion is moderately but significantly elevated \((P<0.0125)\) following chronic iproniazid administration.

![Table 1](image)

Also shown in Table 1 are the effects of L-dopa and 5-HTP administration on the prolactin levels in the chronic iproniazid treated rats. Both L-dopa and 5-HTP caused a significant \((P<0.025)\) suppression of prolactin secretion in these animals.

![Table 2](image)

With the exception of the group of rats administered L-dopa (which were not examined further since the ability of L-dopa to elevate brain DA is well established) the concentrations of DA, NA and 5-HT in the hypothalamus and anterior pituitary of the controls and groups following chronic MAOI are shown in Table 2. In contrast...
to the effects seen after an acute single dose of iproniazid, the levels of DA in the hypothalamus following chronic iproniazid administration were not significantly different from controls. However, both NA and 5-HT were highly significantly elevated ($P<0.0005$) in the hypothalamus to levels approximately double those of controls. The concentrations of DA and NA in anterior pituitary tissue were very low and these levels did not differ significantly between the test groups and controls. However, the concentration of 5-HT in the pituitary was significantly elevated ($P<0.0125$) by chronic iproniazid. The effects seen in the median eminence following chronic iproniazid administration (shown in Table 2) were similar to those in the hypothalamus except that in the case of this tissue DA concentrations were significantly ($P<0.05$) lower than in controls. The administration of 5-HTP to the iproniazid-treated animals caused a slight, but not significant, further increase in 5-HT concentrations in the tissues examined.

Discussion

The results of this investigation confirm the earlier findings of Lu and Meites (1971) with respect to the effects of acute administration of the MAOI iproniazid on rat prolactin secretion. The elevated hypothalamic DA concentrations observed in this situation support the proposal that the inhibitory effect on prolactin is due to increased hypothalamic DA activity (Lu and Meites 1971). However, it is clear that following chronic administration of iproniazid the effects on both prolactin secretion and the hypothalamic–median eminence DA concentrations differ markedly from the situation following a single dose. After chronic iproniazid treatment prolactin secretion was significantly elevated—a result in good agreement with the findings of Slater et al. (1977) with respect to chronic MAOI therapy in humans.

The mechanism whereby prolactin secretion differs markedly between acute and chronic treatment with MAOI can be clearly related to the different status of hypothalamic–median eminence DA in the two cases. The possibility of a prolactin stimulatory effect of raised 5-HT levels following MAOI alluded to by Slater et al. (1977) appears unlikely in view of the effect of 5-HTP administration on prolactin secretion in the chronic iproniazid rats. 5-HTP caused a highly significant suppression of prolactin in these animals which was similar to the (expected) suppression seen following L-dopa. In normal rats, the dose of 5-HTP used in this study has no significant effect on prolactin secretion. It is not clear why 5-HTP has an inhibitory effect on prolactin in chronic iproniazid treated rats but it is possible in this case that the additional 5-HT is able to exert an agonistic effect at DA receptors, or else it has the ability to increase the sensitivity of DA receptors.

The reason why hypothalamic–median eminence DA concentration which is first elevated by MAOI, then falls, following chronic treatment, is presumably due to a reduction in the rate of DA synthesis. This might be brought about by auto-reduction mediated through presynaptic DA receptors. While feedback regulation of neuronal amine synthesis is generally considered quite specific, it is possible to speculate that the high levels of NA might reduce neuronal DA synthesis through a negative feedback process. However, while DA levels are near normal following chronic iproniazid treatment, NA concentrations remain as high as those achieved acutely and it seems that some highly specific mechanism is affecting DA synthesis. Apart from DA auto-reduction mentioned above, another possible reason for specific
reduction in the rate of DA production may lie in an ability of prolactin itself to regulate DA synthesis. It is known that high levels of prolactin secretion result in negative feedback onto the tuberoinfundibular DA neurons of the median eminence to increase DA turnover (Hökhfelt and Fuxe 1972). Perhaps the acute suppression of prolactin after MAOI results in reduction of DA synthesis in order to maintain basal secretion of prolactin. A complicating factor in these considerations is the possible stimulation of prolactin secretion by the high concentrations of hypothalamic-median eminence NA (Donoso et al. 1971).

It is unlikely that effects observed in this study are due to direct effects of the drugs at the level of the pituitary gland in view of previous work showing a lack of effect of MAOI on prolactin secretion from pituitary cells in tissue culture (Abeles and Tashjian 1974). Furthermore, in the present study the pituitary concentration of DA was not significantly altered between treatments. While 5-HT concentration in the pituitary was enhanced following iproniazid, previous studies have shown that 5-HT has no direct effects on the pituitary to alter prolactin secretion (McLeod and Lehmeyer 1972).

The data of this investigation demonstrate that a quite specific change occurs in the regulation of hypothalamic DA when iproniazid is administered chronically. This effect could not have been predicted from knowledge of the acute effects of iproniazid on brain monoamines but changes in serum prolactin correlate well with alterations in the status of hypothalamic DA. These results emphasize the need for caution when conclusions about the chronic effects of drugs on brain biogenic amine function are drawn from data relating only to their acute effects.

References


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