INHIBITION OF THE RELEASE OF CORTICOTROPHIN FROM THE HYPOPHYSIS BY CHLORPROMAZINE

By

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The initial purpose of the experiments reported in this paper was to investigate whether surgical hypophysectomy necessary for the testing of corticotrophin according to Sayers, Sayers & Woodbury (1948) could be replaced by a pharmacological blockade of the corticotrophin-release of the gland by the administration of chlorpromazine. Blockade of diencephalic centres by depressant drugs might also provide more knowledge about the role of the hypothalamus in pituitary function. Chlorpromazine, one of the phenothiazine derivatives which are now widely used to prevent autonomic activity, was chosen since it possesses such a central depressant action. Hiebel et al. (1954) have shown that chlorpromazine suppresses »the central activating effects of adrenaline and also the disturbances activated through the reticular formation as a result of sensory or nociceptive stimuli«. Aron et al. (1953) and Castaigne (1954) observed a complete inhibition of the release of corticotrophin by chlorpromazine treatment. This effect was assumed to be due to suppression of hypothalamic activity. Holzbauer & Vogt (1954), however, could not find any pituitary or hypothalamic inhibition of this drug, whereas Georges & Cahn (1953) even described a corticotrophic effect.

In the present work evidence has been obtained that chlorpromazine inhibits the release of corticotrophin which normally occurs during systemic stress.

MATERIAL AND METHODS

Male and female albino rats of an inbred strain were used. The bodyweight varied between 120-160 gm. The rats were kept at an environmental temperature of 22°C and fed ad libitum on a standard diet.

It is well known that the handling of normal rats is followed by a fall in the ascorbic acid content of the adrenals. In order to avoid this as much as possible the
rats were accustomed to the experimental procedure beforehand by daily injection of sodium pentobarbital during the three days preceding the actual experiment. Pentobarbital was used as an anaesthetic to avoid pain reactions caused by the administration of chlorpromazine. The possibility that pentobarbital in some way interferes with the action of chlorpromazine will be discussed later.

On the experimental day the rats were anaesthetized by the intravenous administration of pentobarbital (males 3.0 mg., females 3.25 mg./100 gm. bodyweight). After twenty minutes 1 mg. chlorpromazine per 100 gm. (Specia) was given intravenously and after another twenty minutes the same amount of chlorpromazine was administered intramuscularly. This was done in order to obtain a rapid and prolonged action of chlorpromazine.

The pituitary function was tested by measuring the ascorbic acid depletion of the adrenals after the application of different kinds of stimuli. Ascorbic acid was determined according to Roe & Knether (1943) as modified by Sayers et al. (1948).

A. Ascorbic acid depletion of the adrenal after unilateral adrenalectomy.

On the day of the experiment a number of female rats were divided into six groups and injected with pentobarbital and chlorpromazine at various intervals according to the scheme mentioned above. The left adrenal was removed 0, 1, 2, 4, 8, and 24 hours respectively after the last injection. If the action of pentobarbital had subsided the animals were further anaesthetized for this operation with ether.

The right adrenal was removed one hour after the left one. The »difference« between the left and the right adrenal was taken as an index of pituitary activity. As it was found that the maximal effect of chlorpromazine was obtained if the left adrenal was removed 1–2 hours after the treatment with this substance, a second experiment was performed with a time interval of one hour for all the animals. This experiment was performed with male as well as female rats, the controls receiving sodium pentobarbital only.

B. Ascorbic acid depletion of the adrenals after treatment with systemic stimuli.

Since it is known that the adrenal reaction during several kinds of stresses may be abolished by pentobarbital anaesthesia (Recant et al., 1950) we used adrenaline, nor-adrenaline and histamine as stressor agents. These substances are known to cause an adrenal ascorbic acid depletion even in rats anaesthetized with pentobarbital (Kenskamp, 1955; Briggs & Munson, 1955).

Pentobarbital and chlorpromazine were administered to groups of male as well as female rats in the manner described above. A control group received pentobarbital only. One hour after this treatment the animals were injected intraperitoneally with adrenaline, nor-adrenaline, histamine or saline. One hour after this second treatment the rats were sacrificed, both adrenals were removed and their ascorbic acid content determined (Munson’s modification of the method by Sayers et al.). The difference in the ascorbic acid content of the adrenals between the animals treated with a stressor agent and that of the rats treated with saline served as an index of pituitary activity.

C. The effect of different doses of corticotrophin injected intravenously on the ascorbic acid content of the adrenals in rats pretreated one hour previously with pentobarbital and chlorpromazine was also studied. The latter substances were administered in the same way as mentioned above. Hypophysectomized rats were prepared by the parapharyngeal approach and were used in the adrenal ascorbic acid depletion test 20–24 hours after hypophysectomy.
The statistical analysis of all the results was performed with the aid of Wilcoxon's two sample test (Wilcoxon, 1945; Mann & Whitney, 1947; van der Vaart, 1950). A difference was considered as statistically significant, if the double tail probability $= < 0.05$.

RESULTS

In preliminary experiments the chlorpromazine was administered subcutaneously. Only a small inhibition of the pituitary function was found when 1 or 2.5 mg. were injected one hour before testing the pituitary-adrenal reaction. Moreover the results were rather variable and the adrenal ascorbic acid content was low even before the application of any stressor agent. This could not be improved by changing the amount of chlorpromazine, by varying the time between the injection of the inhibitor and the stressor agent or by repeated chlorpromazine administration. The low initial ascorbic acid content might be explained by the assumption that the very painful subcutaneous injection of chlorpromazine was followed by a corticotrophin discharge before the drug was able to inhibit the pituitary function. In order to get a more rapid inhibitory action and to diminish the pain we then administered the chlorpromazine intravenously, but the results were not much better. Subsequently pentobarbital was given prior to the chlorpromazine treatment in order to prevent the pain, and the chlorpromazine was injected both intravenously and intramuscularly. When this more complicated scheme of administration was adopted, more satisfactory initial values were obtained as can be seen from Fig. 1. The administration of chlorpromazine still caused a decrease in ascorbic

![Graph showing ascorbic acid content of left and right adrenals at different times after treatment with chlorpromazine + pentobarbital.](image)

*Fig. 1.*

Ascorbic acid content of left and right adrenals at different times after treatment with chlorpromazine + pentobarbital.

The number of rats in each group is given between parentheses.
acid content, but during the 1–2 hours only to a limited extent. The effect was not statistically significant.

A. The diagram also shows that after the injection of chlorpromazine the stress caused by the ablation of one adrenal is still followed by a fall in the vitamin C content of the gland. However, this fall was less during the 1st–8th hour, i.e. during the period in which chlorpromazine may be assumed to develop its strongest action, than it was after 24 hours, at which time the effect of the drug is almost certainly over. From this it may be concluded that chlorpromazine partly prevents the ascorbic acid depletion caused by unilateral adrenalectomy. After one hour, the inhibition was found to be at its maximum.

In the second experiment of this series in which the period of one hour between the last injection and the operation was the same in all rats, the inhibitory effect of chlorpromazine was far more marked than in the first one (Table 1). After the administration of chlorpromazine the unilateral adrenalectomy caused a fall of 3.25 mg. of ascorbic acid. However, the inhibitory effect of chlorpromazine was far more marked than in the first one (Table 1).

Table 1.
The effect of pretreatment with pentobarbital and chlorpromazine on the adrenal ascorbic acid depletion after unilateral adrenalectomy.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Pretreatment</th>
<th>Ascorbic acid content of left minus right adrenal mg./100 gm. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sodium-pento-barbital i. v.</td>
<td>chlorpromazine i. v.</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>♀</td>
<td>3.25*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♀</td>
<td>3.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>3.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*) all doses per 100 gm. bodyweight.
Table 2.
The effect of pretreatment with pentobarbital + chlorpromazine or with pentobarbital alone on the adrenal ascorbic acid depletion caused by stress.

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Sex</th>
<th>Pretreatment</th>
<th>Stressor agent</th>
<th>Adrenal ascorbic acid content&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>P. Wilcoxon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>sodium-pento-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>barbital i. v.</td>
<td>chlor-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>promazine i. v.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>chlor-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>promazine i. m.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>♀</td>
<td>3.25&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>1</td>
<td>adrenaline, 30 µg. i. p.</td>
<td>404 ± 9.1</td>
</tr>
<tr>
<td>13</td>
<td>♀</td>
<td>3.25</td>
<td>1</td>
<td>saline, ½ ml. i. p.</td>
<td>415 ± 12.7</td>
</tr>
<tr>
<td>12</td>
<td>♀</td>
<td>3.25</td>
<td>1</td>
<td>histamine phosphate, 5 mg. i. p.</td>
<td>409 ± 10.6</td>
</tr>
<tr>
<td>16</td>
<td>♀</td>
<td>3.25</td>
<td>0</td>
<td>adrenaline, 30 µg. i. p.</td>
<td>372 ± 7.25</td>
</tr>
<tr>
<td>12</td>
<td>♀</td>
<td>3.25</td>
<td>0</td>
<td>saline, ½ ml. i. p.</td>
<td>395 ± 7.37</td>
</tr>
<tr>
<td>16</td>
<td>♀</td>
<td>3.25</td>
<td>0</td>
<td>histamine phosphate, 5 mg. i. p.</td>
<td>311 ± 7.1</td>
</tr>
<tr>
<td>12</td>
<td>♀</td>
<td>3.25</td>
<td>1</td>
<td>noradrenaline bitartrate, 300 µg. i. p.</td>
<td>397 ± 9.04</td>
</tr>
<tr>
<td>13</td>
<td>♀</td>
<td>3.25</td>
<td>1</td>
<td>saline, ½ ml. i. p.</td>
<td>387 ± 7.9</td>
</tr>
<tr>
<td>10</td>
<td>♀</td>
<td>3.25</td>
<td>0</td>
<td>noradrenaline bitartrate, 300 µg. i. p.</td>
<td>330 ± 7.4</td>
</tr>
<tr>
<td>10</td>
<td>♀</td>
<td>3.25</td>
<td>0</td>
<td>saline, ½ ml. i. p.</td>
<td>375 ± 5.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>) mg./100 gm. adrenal gland ± standard error of the mean.
<sup>b</sup>) all doses per 100 gm. bodyweights.
ectomy was followed by a rise in the ascorbic acid level in the majority of the animals, whereas after administration of pentobarbital only a decrease was observed. This applies both to male and female animals.

B. The results of the experiments in which drugs were used as stressors are recorded in Table 2. The adrenal ascorbic acid content of the female rats treated with pentobarbital only, showed a statistically significant decrease after the injection of the chemical stressor agents. If both pentobarbital and chlorpromazine were given to females none of the substances used, i.e. histamine, adrenaline and nor-adrenaline, caused a decrease. This means that chlorpromazine is able to prevent the stimulation of the pituitary-adrenal system by histamine, adrenaline and nor-adrenaline.

C. The data concerning the ascorbic acid level of the adrenals after treatment with corticotrophin in rats pretreated with chlorpromazine and pentobarbital are presented in Fig. 2. By plotting the ascorbic acid content against the log-dose a straight line was obtained. Each point is an average of the data for 11-15 rats. From this figure it is seen that the ascorbic acid depletion is small, 0.25 µg. of corticotrophin giving a depletion of only 53 mg./100 gm. adrenal (13%). This might be due either to an inhibition of the adrenal response to corticotrophin by chlorpromazine or to the fact that the initial adrenal ascorbic acid level was rather low. It is known that in the «Sayers test» the decrease in the vitamin C-content is almost proportional to the initial adrenal ascorbic acid level. To distinguish between the two possibilities, two groups of hypophysectomized male rats were injected, one group with 0.25 µg. corticotrophin and another group with 0.25 µg. corticotrophin and 1 mg. chlor-

![Diagram](image_url)

**Fig. 2.**

Curve obtained by plotting different doses of corticotrophin against the adrenal ascorbic acid content, one hour after corticotrophin injection. Numbers in brackets represent the number of rats used.
promazine per 100 gm. bodyweight. The mean ascorbic acid depletion was 97 ± 7.5 (18%) and 90 ± 2.7 (17%) respectively. This difference is not statistically significant, so that the adrenal response to corticotrophin does not appear to be influenced by treatment with chlorpromazine. Hence the small depletion observed in rats receiving 0.25 μg. of corticotrophin appears to be due to the low initial level of the ascorbic acid content of the adrenal gland.

DISCUSSION

Our results show that chlorpromazine prevents at least partly the release of corticotrophin brought about by histamine, adrenaline, nor-adrenaline or unilateral adrenalectomy. These findings confirm the results reported by Aron et al. (1953) and Castaigne (1954), who found no depletion of the adrenal ascorbic acid either after removal of one adrenal gland or following an injection of formalin, and they are contrary to those of Holzbauer & Vogt (1954), who described an ascorbic acid depletion after the administration of adrenaline in rats treated with chlorpromazine.

It is unlikely that the effect of chlorpromazine on the pituitary activity caused by the substances used as systemic stimuli, resulted from a specific antagonism to these drugs. With regard to histamine Courvoisier et al. (1953) have shown that chlorpromazine has only a very slight antihistaminic action. The sympatholytic property of chlorpromazine too cannot be held responsible for the inhibition of the effect of sympathomimetics. This assumption is based on the fact that chlorpromazine has a marked adrenolytic effect but only a very slight noradrenolytic activity (Huidrobo, 1954; Marquardt et al., 1955), whereas the effect of nor-adrenaline on the hypophysis is abolished by chlorpromazine to the same extent as that of adrenaline.

In our experiments chlorpromazine partly blocks the release of corticotrophin after unilateral adrenalectomy. Sayers & Sayers (1948) have postulated that the hypophysis is activated by a reduction in the concentration of corticoids in the circulation. As chlorpromazine depresses cellular activity (Decourt & Anguera, 1953), it might be possible that in the presence of this substance the consumption of circulating adrenal cortical hormones is diminished. Hence the secretion of corticotrophin might be partly inhibited as a consequence of the higher level of corticoids in the blood.

A depression of cellular activity might also occur in the hypophysis. In fact this may be another mechanism by which chlorpromazine exerts its blocking effect on the pituitary gland. In accordance with this possibility is the report by Georges & Cahn (1953), who observed that pituitary inhibition after treatment with chlorpromazine occurs in animals in which the body temperature was decreased to 27°C. Under such conditions a depression of the metabolism of susceptible tissues may be assumed to exist. No definite conclusions can be
drawn since in our experiments the body temperature was not determined. The function of the adrenal gland was not found to be directly influenced by chlorpromazine since ascorbic acid depletion after administration of corticotrophin was the same in chlorpromazine treated and in untreated rats.

Hiebel et al. (1954) have shown that chlorpromazine has a central depressant action. In recent years much work has been done on the relation between the hypothalamus and the hypophysis. Harris & Jacobsahn (1952) have postulated that the release of corticotrophin is mediated by a substance of hypothalamic origin. Stimulation of selected areas in the hypothalamus causes secretion of corticotrophin (de Groot & Harris, 1950; Hume, 1952; Porter, 1953), while electrolytic lesions in these centres inhibit the release of corticotrophin during stress (McCann, 1953; Hume, 1952; Porter, 1953). These experiments strongly suggest that the secretion of corticotrophin during stress depends on a normal hypothalamic function. Hence a further explanation for the effect of chlorpromazine might be a depressant action on these hypothalamic centres.

As already mentioned in preliminary experiments subcutaneous injection of small doses of chlorpromazine was rather painful and caused a decrease in the adrenal ascorbic acid content. In order to abolish the pain and to get a more rapid effect of the drug, chlorpromazine was injected intravenously, but the same low values were found. From these experiments it may be concluded that chlorpromazine stimulates the adrenal gland in normal rats before the postulated inhibition of the pituitary gland occurs. This is in agreement with the findings of Georges & Cahn (1953) who described corticotrophin-like activity of chlorpromazine. In our experiments pentobarbital was used in order to prevent the stimulating effect of chlorpromazine. The question may arise whether the blockade observed was due to the action of chlorpromazine alone or to the combined effect of chlorpromazine and pentobarbital. The investigations of Aron et al. (1953) and Castaigne (1954), which were carried out without giving an anaesthetic drug together with chlorpromazine, suggest that pituitary inhibition is due to the effect of the latter substance alone.

The ascorbic acid level after treatment with chlorpromazine and using unilateral adrenalectomy as a stressor agent was found to depend on the time of the day at which the experiments were performed. In the experiments carried out in the morning an increase of the ascorbic acid level was observed, whereas in those which were done in the afternoon the operation was followed by a decrease. It is known that the ascorbic acid level of the adrenals in rats rises during the day and falls during the night. Hence the increase after the operation in the morning might coincide with the diurnal rise of the vitamin-level in the adrenal, whilst the decrease following the operation in the afternoon might be explained by the fact that during the experiment the basic ascorbic acid content in the adrenal gland has reached its maximum or was already falling.
The adrenal response to corticotrophin was more pronounced in hypophysectomized than in normal rats treated with chlorpromazine. The decrease of adrenal ascorbic acid is known to be proportional to the initial value. The difference in response is presumably due to the fact that the initial ascorbic acid values in hypophysectomized rats are much higher than those in the non-hypophysectomized animals treated with chlorpromazine.

A functional hypophysectomy by chlorpromazine and pentobarbital in order to obtain animals in which corticotrophin determinations can be made, does not appear of much practical use for the following reasons:

1. there is no proof that chlorpromazine has a direct inhibitory action on the hypophysis.
2. the ascorbic acid content of the adrenal gland after treatment with chlorpromazine and pentobarbital is rather low; this means a less sensitive test object than the hypophysectomized rat with its high initial values.
3. the combined treatment with intravenous and intramuscular injections is rather complicated.

SUMMARY

The effect of administration of chlorpromazine on the secretion of corticotrophin was investigated. An injection of adrenaline, histamine or nor-adrenaline caused an ascorbic acid depletion of the adrenals in control rats pretreated with pentobarbital, but not in rats which were given both chlorpromazine and pentobarbital.

The same treatment blocked the ascorbic acid response to unilateral adrenalectomy in the remaining adrenal gland for at least two hours. Partial inhibition was observed during the following six hours.

After administration of corticotrophin there was no significant difference between the adrenal ascorbic acid depletion of hypophysectomized rats treated with chlorpromazine and pentobarbital and those only anaesthetized with pentobarbital. This means that the site of action of chlorpromazine is not the adrenal gland.

It was concluded that chlorpromazine inhibits the release of corticotrophin which normally occurs during stress. For several reasons the pituitary inhibition induced by chlorpromazine can not be considered as a substitute for hypophysectomy.

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