The Ability of Perindopril of Modulate Behavioural, Neuroendocrinal and Testicular Profiles in Stressed Rats

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ABSTRACT. The study was conducted on 42 adult male Wistar rats, receiving a daily IP injection of: 1) 0.2 ml saline, in control group (n = 6). 2) 0.2 ml saline in stress group (S-GP) (n = 18). 3) 400 μg/kg perindopril, in perindopril pretreatment stress group (P-P S-Gp) (n = 18). After 3 weeks, animals in group 2 & 3 were each equally subdivided and subjected to short term modalities of stress: Heat & Light (H&L-S), (n = 6), Cold (C-S), (n = 6) and immobilization & Immersion (I&I-S), (n = 6). The sequelae of these were firstly assessed on some behavioural activities, then on some neuroendocrinal responses and subsidiary testicular histopathological changes, instantaneously after decapitation. Results revealed that P-P H&L-S prevented the significant changes in adrenocorticotropic hormone (ACTH), testosterone and non-social investigations and significantly decreased, but to lesser extent, the elevation in norepinephrine (NE), yet did not prevent the significant alterations in dopamine (DA), cortisol and displacement, induced by H&L-S, in comparison to control. Meanwhile, this pretreatment increased significantly the social investigations and 5HT in comparison to control. On the other hand, P-P C-S prevented the significant changes in NE and threat induced by C-S, yet it significantly increased social investigations, in comparison to control. However, P-P I&I-S prevented the significant changes in prolactin (PRL) and non social investigations and significantly decreased, to a lesser extent, the changes in NE, DA, ACTH, cortisol, testosterone and defence, but pertained the significant elevation in 5HT, induced by I&I-S, in comparison to control. Meanwhile, this pretreatment significantly increased social investigations and decreased displacement in comparison to control. No testicular histopathological changes were inflicted by S-Gps or modulated by P-PS Gps.

Introduction

Studied probing in the impacts of stress on psyche and soma are still a dilemma without solid links. On epidemiological basis, the role of stress as a segregated etiological factor
in different psychological cardiovascular, endocrinal and metabolic disorders and disturbances have long been raised\[1-3\]. However, when translated into biochemical terms; the psychoemotional provocation aroused by wear and tear insults, that are evoked by different threatening physical and/or psychological stresses appear up till now to intricate nature\[2,4\]. Though, a common non specific hypothalamic-pituitary-adrenocorticomedullary release and sympathetic nerve terminal stimulation, \textit{i.e.}, what is termed neuroendocrinal response to stress, has long been coined\[2-5\]. But, whether or not the varied stressors, are able to provoke the release of the same synaptic and humoral components of this response and with the same intensity, is still argued and needs to be explained\[4,6,7\]. Furthermore, only few studies have demonstrated an etiopathophysiological link between all forestated variants and the intersecting hypothalamic-pituitary-testicular axis, to influence male fertility, a point that needs to be thoroughly highlighted.

The present study, thus focuses on the impacts on three modalities of stress, linking their related behavioural alterations to the probable relevant simultaneous fluctuations in brain neurotransmitters, pituitary and steroid hormones and histopathological alterations in the testis of male rats.

In addition, the study also examines a drug such a perindopril [P] (Coversyl, Servier LTD. French\[8\]) a novel angiotensin converting enzyme inhibitor, classically administered to control hypertension \[where stress per se plays a relevant etiological precipitating factor]\[3\]. It questions; whether or not this drug can favourably ameliorate the varied impacts of stress tackled in this study, as efficiently as, when controlling the hypertensive outcome of stress\[8\]. If so is the case, then recognizing drugs for use that can strike at multiple levels to curtail the hazardous outcomes of stress on psyche and soma would be an important target to achieve.

**Experimental Procedures**

42 adult male Wistar rats [170-200 g] were reared under controlled conditions of temperature and reversal light cycle. Food and water were added ad libitum. After one month of being acclimatized, animals were then divided into three groups which received a daily IP injection of (a) 0.2 ml saline in the control group (n = 6), (b) 0.2 ml saline in the stress group [S-Gp] (n = 18), and (c) 400μg/kg P(8) [Coversyl - Servier LTD. French] in perindopril treatment stress group [P-P S-Gp] (n = 18). Animals were kept under the same standard conditions for three more weeks after which those of groups (2) and (3) were further equally subdivided into three relevant subgroups, each of which being subjected to one of the short term modalities of stress experimented namely:

**Heat and Light Stress [H&L-S];** where the top of the cage was continuously eliminated for 36hr by 200V lamp; thus breaking the light cycle and conferring thermal insult throughout\[9\].

**Cold Stress [C-S];** where rats were refrigerated for 12hr at 4°C\[10\].

**Immobilization and Immersion Stress [I&I-S];** where the fore and hind limbs of each rat were tied up to and locked in a small metal cage, that was immersed in cold water 4°C for 24hr, while sparing its head out\[11\].
Ethological Behavioural Assessments

Once stress experimentations were terminated, each animal from the three studied groups was placed into a special partitional cage against its standard opponent[12] and the broad behavioural categories, *i.e.*, social, non-social investigations, attack, threat, defence and displacement as well as the number of actual attacks were all recorded over 900 seconds using coloured video camera[13]. The tapes were then analysed for allocating the time consumed in each behavioural category[12,13]. These data were statistically analysed using Kruskal-Wallis test with appropriate comparison being made using Mann-Whitney "U" test[14].

Analytical Biochemical Estimates

After terminating the previous testing, each animal was decapitated, the blood was instantaneously collected and the brain was simultaneously frozen in liquid nitrogen once dissected. These samples were used to determine:

*Neurotransmitters*: where the whole brain was weighted and homogenised to determine norepinephrine (NE), dopamine (DA) and serotonin (5HT) by the fluorometric micromethod[15] using an Aminco Bowman fluorospectrophotometer.

*Hormones*: where serum ACTH, PRL and testosterone levels were determined by a RIA technique[16-18] and serum cortisol level was determined by an ELISA technique[19].

All biochemical data were statistically analysed using the student (t) test for unpaired comparison[20].

Histopathological Changes

The testes were removed from all studied animals, the epididymis was dissected out and the testicular tissue was impregnated in Buan solution then Formalin, processed, sectioned and stained by H&E to detect any structural changes least they have developed[21].

Results

**Behavioural Changes; Demonstrated in Table 1 and Figure (1)**

The time allocated to non-social investigation was significantly prolonged and that to displacement was significantly by H&L-S in comparison to control. P-P H&L-S, significantly shortened the time advocated to threat in comparison to its relevant H&L-S and pertained that of displacement shorted in comparison to control. The time consumed in threat was significantly decreased in C-S and I&I-S, while that of non-social investigation was significantly shorted and that of defence attempt was significantly prolonged only in I&I-S all in comparison to control. P-P I&I-S significantly shortened time allocated to non-social investigation, defence and displacement in comparison to relevant I&I-S, the latter attempt, being also significantly shortened in comparison to control. In all P-P S-Gps, the time consumed in social investigation was significantly prolonged whether in comparison to control or to their respective S-Gps.
Whole Brain Contents of Monamines Demonstrated in Table 2 and Figure (2)

NE and DA were significantly increased by H&L-S and tended to decrease by P-P H&L-S, though still being considered significantly elevated, when compared to control. However, NE induced decrease, was significant when comparing P-P H&L-S to relevant H&L-S alone. On the contrary, this drug regimen induced a significant increase in 5HT in comparison to control. In C-S only NE was significantly elevated and P-P to C-S protected against the rise, but meanwhile, induced a significant elevation in 5HT in comparison to control. In I&I-S all studied amines were significantly elevated while 5HT was pertained significantly elevated but NE and DA tended to decreased by P-P I&I-S though were still considered elevated in comparison to control.

Table 1. Ethological behavioural changes in rats subjected to three different modalities of stress per se or after three weeks pretreatment with perindopril (400 mg/kg) daily IP. (Value are expressed as Median & Range and compared by using Mann-Whitney “U” test).

<table>
<thead>
<tr>
<th>Behavioural assessment studied Gps</th>
<th>Time allocated to ethological changes in seconds</th>
<th>Social investigation</th>
<th>Non social investigation</th>
<th>Attack</th>
<th>Threat</th>
<th>Defence</th>
<th>Displacement</th>
<th>No. of attacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Gp. (n = 6)</td>
<td></td>
<td>202.5 [65 - 280]</td>
<td>280.0 [150 - 395]</td>
<td>0.0 [0.0 - 0.0]</td>
<td>25 [10 - 40]</td>
<td>0.0 [0.0 - 10]</td>
<td>13.0 [35 - 215]</td>
<td>0.0 [0.0 - 0.0]</td>
</tr>
<tr>
<td>Stress Gps.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) H&amp;L-S (n = 6)</td>
<td></td>
<td>54.0 [44 - 200]</td>
<td>410** [228 - 488]</td>
<td>0.0</td>
<td>36.0</td>
<td>0.0</td>
<td>30.0 [24 - 108]</td>
<td>0.0 [0.0 - 8.0]</td>
</tr>
<tr>
<td>b) C-S (n = 6)</td>
<td></td>
<td>189.0 [68 - 489]</td>
<td>289.0 [105 - 475]</td>
<td>0.0</td>
<td>0.0***</td>
<td>0.0</td>
<td>57.0 [0.0 - 122]</td>
<td>0.0 [0.0 - 0.0]</td>
</tr>
<tr>
<td>c) I&amp;I-S (n = 6)</td>
<td></td>
<td>115.0 [75 - 150]</td>
<td>65.5*** [45 - 160]</td>
<td>0.0</td>
<td>0.0***</td>
<td>215.0***</td>
<td>215.0 [105 - 240]</td>
<td>0.0 [0.0 - 0.0]</td>
</tr>
<tr>
<td>P-P S-Gps.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) P-P H&amp;L-S (n = 6)</td>
<td></td>
<td>569.0***+++ [254 - 781]</td>
<td>269.5 [68 - 621]</td>
<td>0.0</td>
<td>0.0++</td>
<td>0.0</td>
<td>41.5** [9 - 92]</td>
<td>0.0 [0.0 - 0.0]</td>
</tr>
<tr>
<td>b) P-P C-S (n = 6)</td>
<td></td>
<td>607.5***+++ [477 - 769]</td>
<td>376.5 [53 - 357]</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>61.5 [49 - 82]</td>
<td>0.0 [0.0 - 0.0]</td>
</tr>
<tr>
<td>c) P-P I&amp;I-S (n = 6)</td>
<td></td>
<td>645.0***+++ [465 - 719]</td>
<td>239.0+++ [170 - 413]</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0+++</td>
<td>12.5***+++ [9 - 60]</td>
<td>0.0 [0.0 - 0.0]</td>
</tr>
</tbody>
</table>

***; (P < 0.001), **; (P < 0.01), *; (P < 0.05) in comparison to Control Gp.
+++; (P < 0.001), comparing Perindopril Pretreatment Stress Gp. (P-P S-Gp) to relevant Stress Gps.
Heat and Light Stress: (H&L-S) / Cold Stress: (C-S) / Immobilization & Immersion Stress: (I&I-S).

Hormonal Changes; Demonstrated in Table 3 and Figure (3)

Serum ACTH and cortisol, were significantly increased and testosterone was significantly decreased by H&L-S, in comparison to control. This profile was controlled by P-P H&L-S except for cortisol that persisted elevated in comparison to control. I&I-S significantly decreased testosterone and increased the rest of hormones in comparison to control. P-P I&I-S tended to confer little protection; as this profile, was pertained but at a lesser degree of significance except for PRL that reverted back to control. Thus PRL and cortisol were found significantly decreased by this regimen, when compared to I&I-S.
The Ability of Perindopril to...

Fig. 1. Behavioural conduct changes in rats subjected to different modalities of stress with or without perindopril pretreatment.

**Table 2.** Changes in brain neurotransmitter concentration of rats subjected to three different modalities of stress per se or after three weeks pretreatment with perindopril (400 µg/kg) daily IP.

(Values are expressed as Mean ± SE.) and compared using Student “t” test.)

<table>
<thead>
<tr>
<th>Studied Gps</th>
<th>Control Gp</th>
<th>H&amp;L Gps (n=6)</th>
<th>Stress Gps (n=6)</th>
<th>P-Pretreatment stress Gps (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain monamines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE in µg/g. wet brain tissue</td>
<td>0.6521</td>
<td>± 0.0711</td>
<td>1.9657***</td>
<td>0.8789*</td>
</tr>
<tr>
<td>DA in µg/g. wet brain tissue</td>
<td>1.7642</td>
<td>± 0.0928</td>
<td>2.6965*</td>
<td>2.0921</td>
</tr>
<tr>
<td>SHT in µg/g. wet brain tissue</td>
<td>0.6739</td>
<td>± 0.0573</td>
<td>0.8929</td>
<td>0.9432</td>
</tr>
</tbody>
</table>

***: (P < 0.001), **: (P < 0.01), *: (P < 0.05) in comparison to Control Gp – Perindopril Pretreatment Stress Gps (P-P S-Gps).

Heat & Light Stress: (H&L-S) / Cold Stress: (C-S) / Immersion & Immobilization Stress: (I&I-S).

+++; (P < 0.01), +++; (P < 0.001), when comparing Perindopril Pretreatment Stress Gps. (P-P S-Gps) to relevant Stress Gps. Heat and Light Stress: (H&L-S) / Cold Stress: (C-S) / Immersion & Immobilization Stress: (I&I-S).
FIG. 2. Brain monamines’ changes in rats subjected to different modalities of stress with or without perindopril pretreatment.

TABLE 3. Hormonal changes in rats subjected to three different modalities of stress per se or after three weeks pretreatment with perindopril (400 μg/kg) daily IP.

<table>
<thead>
<tr>
<th>Hormonal levels</th>
<th>Pituitary hormones</th>
<th>Steroid hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACTH pg/ml</td>
<td>Prolactin uUL</td>
</tr>
<tr>
<td><strong>Control Gp.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td>1102.2</td>
<td>± 112.4</td>
</tr>
<tr>
<td><strong>Stress Gps.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) H&amp;L-S</td>
<td>1464.7*</td>
<td>± 108.6</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td>± 211.3</td>
</tr>
<tr>
<td>b) C-S</td>
<td>1328.5</td>
<td>158.2</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td>± 114.6</td>
</tr>
<tr>
<td>c) I&amp;I-S</td>
<td>1592.3**</td>
<td>± 104.6</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P-P S-Gps.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) P-P H&amp;L-S</td>
<td>1372.1</td>
<td>± 99.7</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td>± 268</td>
</tr>
<tr>
<td>b) P-P C-S</td>
<td>1234</td>
<td>148.6</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td>± 116.9</td>
</tr>
</tbody>
</table>

***; (P < 0.001), **; (P < 0.01), *; (P < 0.05) in comparison to Control Gp. 
+; (P < 0.05), when comparing Perindopril Pretreatment Stress Gps (P-P S-Gps) to relevant Stress Gps.
Heat and Light Stress: (H&L-S) / Cold Stress: (C-S) / Immersion & Immobilization Stress: (I&I-S).
**Histopathological Changes; Demonstrated in Plate-I**

It illustrates that no structural testicular alterations were detected neither by S-Gps nor relevant P-P to S-Gps, in comparison to control.

**Discussion**

Justifying the validity of the concept that argues the “homogenicity of stressors” versus the “homogenicity of responders” was one of the tasks worthwhile probed. This is to partition whether all stressors evoke similar internal consequences that function the maximize survival in face of external predatory threats[21] or whether each has a particular profile of its own[4].

Answers to this have always been questionable, because difficulties always exist to standardized within the lab insults presenting in daily life. However, just by simultaneously correlating the behavioural and neuroendocrine response outcome to the variable short term modalities of stress adopted in this study, an answer could be conspicuously speculated. Thus responses recorded appeared differential, *i.e.* stressor specific[21,22]. This is an issue now reported in many studies clearing that responses as a whole are both adaptive in nature and patterned to environmental demand[4,22-24].

For instance, when present data are viewed collectively, I7I-S appeared the most offending while C-S was the least. I&I insult comprises a complex of psychologically in-
teracting, component provoked by immobilization and boosted by fear due to the distressing existential element of fright from drowning\(^4\). Such stressful stimuli are abet to evoke immense behavioural and neuroendocrinal alterations\(^2,22\) as currently demonstrated. The response pattern was different and more tamed by H&L-S probably as the psychological component it harbours was less distressing [being basically anxiety provoked by disruption of light-dark cycle\(^9,25\)] and is admixed with a physical component (heat exposure). The least response outcome was presently evoked by the physical stimuli of cold exposure; whereby only brain NE content was increased and threat attempt suppressed in comparison to control.

Focusing on each modality, the suppression of threat by C-S could be viewed as part of the patterning the environmental demand whereby all body resources were reported
to be consumed in enhancing thermal metabolic reactions to restore basal metabolic rate to
prestressor level, rather than being consumed in heightening any aggressive attempt\textsuperscript{[22,26,27]}. Meanwhile the present demonstrable increase in NE contents; though appears
controversial in literature, yet could still be explained secondary to an increase in cate
cholamine sensitizing system triggered by cold exposure. This being mediated via a Ca
calmodulin dependent pathway, that was found to increase DA\textsuperscript{[28]} and may be legible
to increase NE, just as presently observed. The absence of significant changes in 5HT
by C-S were in line with reports probing its effect on different serotonin receptor sub-
sets\textsuperscript{[29]}. The lack of other changes induced by cold exposure was also in accordance
with findings in different literatures even through the exposure time was variable in
such comparative studies\textsuperscript{[27,29,30]}.

Shifting to H&L stress, the non social conducts were increased, reflecting avoidance
\cite{[25]} that prevailed at the expense of displacement. Usually different elements of displacement such as grooming are abet to
predominate in restful states following stress, either as a rebound phenomenon or as a
way to reduce arousal and redress imbalance caused by stressors\textsuperscript{[31,32]}. However, this
was not demonstrable in the present work, although most conditions necessary for its
prevalence were existing. For example, increased displacement has been linked to in-
creased ACTH; as currently observed and more so, for its parent CRH, as has been re-
ported\textsuperscript{[33,34]}. This is believed to be mediated via monaminergic transmission particularly
dopaminergic through stimulation of D2 receptor subsets\textsuperscript{[35,36]} and may even be
through adrenergic or serotonergic\textsuperscript{[34,36,37]}.

Currently speaking, the brain content of the former two transmitters was found pres-
ently elevated, and the increase in their activities were also reported to be associated
with anxiety\textsuperscript{[38,39]} while 5HT content in the current work was significantly altered
which could be viewed to coincide with the anxiogenic predominance in this stress mo-
dality studied and as confirmed in other reports emphasizing a role for serotonergic ag-
onists and serotonin reuptake inhibitors as anxiolytics and antidepressants\textsuperscript{[40,41]}.

The increase in environmental temperature encountered in H&L-S could have been
an added factor to booster avoidance displacement. But this is rather questionable, as in-
creased temperature was reported to markedly enhanced the activity of serotonergic
neurons\textsuperscript{[7,41]} and to induce generalized responses to ACTH\textsuperscript{[42]} to profile that was not
totally fulfilled in the current study and that is debated by recent reports arguing a pos-
sible role for serotonin in thermo-regulation\textsuperscript{[37]}. Furthermore, it is relevant to add, that
sleep deprivartion as a component of the H&L modality studied was reported per se by
some not to play a major role in eliciting any neuroendocrine response\textsuperscript{[43]}.

The concomitant suppression in testosteron observed whether by H&L or by I&I-S
could be appraised as part of the overall role of psychological stress on male re-
production\textsuperscript{[44]}. The main brunt of suppression is likely induced by the simultaneous el-
evation in cortisol, where their reciprocal interrelationship has been coined\textsuperscript{[44,45]}. These
reports, have cleared that the binding of cortisol to its receptors on Leydig cells sup-
presses testosterone synthesis on testicular level\textsuperscript{[45]}, which can explain the current find-
ings. While the possible involvement of LH, the controller of testosterone production, as a precipitating factor of suppression on pituitary level has been controversial in other studies; confirmed by some\cite{46} and denied by others\cite{44}, unfortunately not assessed in this study.

In H&L-S, elevated temperature could have been an additive factor to suppress testosterone, but this was not presently observed as it was shown to be linked to increased PRL\cite{42} that was not found elevated in this modality studied. In line, some reports claimed that PRL does not change in psychological stress while others emphasized on its increase by immobilization and restrain\cite{44,47-49}. This was true in the current I&I series where increased PRL could have been an additive factor behind the more significant suppression in testosterone observed in this modality. This increase was thought to involve tubuloinfundibular dopaminergic and serotonergic pathways\cite{44,50,51}, which seems consistent to the finding that brain contents of both transmitters were found presently elevated by I&I-S. Moreover, immobilization per se was also reported to disrupt testicular steroidogenesis by inducing a state of Leydig cell hyposensitivity to gonadotrophins\cite{44,52}, which leaves open a question that is not as yet settled and warrant assessment. However, it is worthwhile to note that the encountered changes by stress were only functional in some modalities while the testicular structure was totally spared.

Still speaking of I&I-S, the component of fear that prevailed could be viewed as the trigger to the increase in all neurotransmitters presently assessed. In this domain, it was previously stated that exposure to threatening stimuli activates the monoaminergic systems in CNS, though in some articles they linked fear more to serotonin\cite{41,53}, while in other they debated any specific role of this transmitter in stress\cite{37,40}. However, what is agreed upon is that when animals are confronted by dangerous situations the emotional defensive behaviour predominates and such offensive defensive pattern is dictated by the strength of the stressor\cite{6,40,53}. This was vividly observed in this study by the existential element of I&I-S that reset attempt to become withdrawn in defense at the expense of threat and has allowed its hangover even after the insult was terminated. The tendency for displacement to prevail over other elements of conduct was too a surprising finding as grooming was reported in literature to be immediately inhibited by severe stressors\cite{31,32}. However, the likely explanation to this could be attributed to fur moistening that was reported to stimulate this act\cite{31}, to partially takeover.

It is apparent from all fore stated that response profile was specific to each modality and its behavioural neuroendocrinal magnitudes were dictated by the character of the stressor employed. It represents a very complex process and it is this complexity that is the cause of the individual response to stress\cite{4,22}.

At this juncture, it is relevant to clear that if these stimulatory processes to stress are unrestricted, the behavioural, neuroendocrinal, circulatory ... etc. of positive feedback loop would threaten rather than foster homeostasis during stress\cite{54}. Cortisol increase was natures protection to create negative feedback control loop to encompass such response\cite{54}. But with nowadays increase in stress intensity, duration, frequency, predictability, chronicity ... etc.\cite{6} an urge to halt its etiopathologic consequences by exogenous tools, has become mandatory; perindopril being assessed in this study.
There appears that, perindopril pretreatment has fine tuned behavioural deficit in attempts and has promoted beneficial elements of conducts to take over. This was vividly apparent as social investigations were significantly enhanced by the drug irrespective of the stress modality probed. This presents an important protective issue as it follows a pattern consistent with compounds possessing anxiolytic potential. This is because, in part, it correlates well with concomitant significant increase in brain serotonin content regardless of changes pre-induced by respective stress modalities; where interlinks between serotonin modulation and anxiolytic action was previously documented. Moreover, the taming effect of perindopril pretreatment over the observed increase in NE and DA that was induced by the psychological component of I&I and H&L stresses, is considered beneficial, as it partially turned off those defense reactions controlling their overshooting rather than shutting them down, in such modalities.

The effect of perindopril on NE could be logically anticipated, knowing that many reports had observed its release from different brain areas by Ag II, but the situation remains questionable with respect to DA and 5HT. Speaking of DA, its activity in nigro-striatal pathways was reported to decrease with the concomitant decrease in Ag II and/or increase in bradykinin, substance P, enkephalines or neurotensin in the brain as a result of ACE inhibition by captopril. In another study Ag antagonists decreased DA content in amygdala and entorhinal cortex; all of which seem consistent to the present findings; though still others have claimed that interaction of Ag and DA receptors are of intricate nature; the yield would favour the enhancement of some receptors and block of others, dictating the same response.

Shifting to serotonin the picture appeared more bizarre, as Ag antagonists were reported to increase serotonin in amygdala and striatum while on the contrary Ag II was the one found to increase it in hypothalamus, brain stem and pineal gland. In a third study, Ag II reached on the rate limiting enzyme (tryptophane hydroxylase) responsible for serotonin synthesis where it suppressed its synthesis at low Ag concentrations and enhanced it with higher levels, which leaves open an area necessitating through future probing.

Nevertheless, it must be remembered that ACE in the brain does not apply hydrolyses Ag, but does so for other neuropeptides that dictate many behavioural and neuro-endocrinal outcomes elicited. No wonder ACE have come under scrutiny and their ability to improve quality of life, to elevate mood and to improve cognitive functions has been coined. Moreover, they possess predictive anxiolytic potentials based on findings relating Ag II to anxiety that is thought to be mediated through AT1 receptor subsets in amygdala. This is being further confirmed by findings that cleared the ability of Ag antagonist to reverse the behavioural deficits induced by stress and to potentiate the antidepressant effect of imipramine in variable studies.

Another point of interest was the current observation that perindopril pretreatment succeeded to reset pituitary response to I&I and H&L-S at a lower pace. Meanwhile, it partially ameliorated the suppression on testosterone but could not sufficiently curtail cortisol pooling during the forestated stress modalities. It is now established in literature...
that endogenous brain Ag II can modulate formation and release of hypothalamic releasing factors\textsuperscript{62} and that the peptide can also be released from the median eminence directly through the portal circulation\textsuperscript{63,70}. Within the pituitary, Ag II has been localized in gonadotrophes of rats where they were reported in various publications to stimulate the paracrine release of PRL and ACTH via AT1 receptors on lactotrophes and corticotrophes\textsuperscript{62,71} and were also reported to exist by itself along with its synthesizing enzyme in human lactotrophes in PRL adenomas\textsuperscript{72}.

Beyond this, circulating Ag II was reported to act directly on the pituitary or via stimulating other circumventricular organs, \textit{i.e.} its acts on areas outside the blood brain barrier (BBB)\textsuperscript{62}. Still to hold, the existence of anatomical links between Erg receptors and pathways inside the BBB and outside it indicates a close correlation between peripheral and central age system in the brain, this contributes to the regulation of hormonal, circulatory and fluid homeostasis and the control of behaviour\textsuperscript{62,64,70}, as observed in varied literatures that were in line with some of perindopril actions observed in this study. Interesting enough, all raised areas outside the BBB were reported to be targets for ACE inhibitors administered systematically and recent reports have even suggested that this group of drugs to penetrate the brain\textsuperscript{64} but the extent of penetration was argued to differ among individual inhibitors\textsuperscript{64,73,74}.

Nevertheless, all forestated data when contrasted would lend credence to the possible mechanisms whereby perindopril could have impinged over to control PRL and ACTH overshooting when prophylactically given to modulate stress as observed in this study. This fosters the raised question as to whether or not Ag neurons are essential components of the neuronal pathways that mediate ACTH increase and enhance the broad spectrum conditioner roles of PRL during stress\textsuperscript{62,75}. This has been raised as the role of Ag in stress has been a subject of intense discussion and controversies\textsuperscript{62,76}. The utility of ACE is as tools to combat stress remains an open question that has been addressed by perindopril findings in the present work.

The current improvement in testosterone profile encountered by perindopril pretreatment to stress modalities studied is of special interest. This is because it partially coincides with perindopril modulation to pituitary secretion as far as PRL and ACTH were concerned but remain unclear with respect to LH that was not currently assessed here, nor was its relation to brain and pituitary Ag totally settled in other reports\textsuperscript{63,77}. The present study observed lack of appropriate suppression in cortisol which appeared partially dissociated from the concomitant changes in [ACTH] induced by perindopril pretreatment is not clear and needs an explanation. But fortunately, this seemed not to exert negative impacts on testicular steroidogenesis as it did not concomitantly induce a parallel suppression in the testosterone currently assessed. Also the likely possibility that increased brain serotonin content observed by perindopril pretreatment would increase PRL and subsidiary suppress testosterone\textsuperscript{44,51}, was luckily not recorded in the stress series presently probed. These protective issues need further justification although it must be admitted that most literature lack records on adverse effects of ACE-Is on testicular functions which fits with the functional and structural findings linked to perindopril pretreatment in the stressed rats currently studied. This could be more con-
firmed by salient reports that assessed the limited penetration of perindopril through the blood-testicular barrier that was potentiated by the lack of drug to modulate ACE in testicular semineferous tubules, a finding that was also linked to quinalapril.

This study thus precluded that perindopril could be a useful tool to curtail many of the hazardous impacts of stress on psyche and soma by conferring anxiolytic inputs, meanwhile preserving male fertility profile. Further experimental work tempting to unravel more solid links on selective brain areas awaits to be assessed ahead before reproducing such advance in clinical trials then in practice.

References


[69] Martin, P., Antidepressant like effects of DuP 753, a non peptide angiotensin II receptor antagonist in the learned helplessness paradigm in rats. *Quoted from ref. 58.*


قدرة البرنودوبريل على إحداث تغيير في السلوك والتفاعلات العصبية والهرمونات وأنسجة الخصبة في الفئران المتوتر

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المستخلص: قمت الدراسة على أثنتين وأربعين ذكرًا من الفئران البالغة، وقد تم حفظها في الفضاء البريتو في ب日に على النحو التالي: المجموعة الأولى: 290 مل من محلول الملح واعتبرت هذه المجموعة هي المجموعة الضابطة وكان عددها ستة فئران. المجموعة الثانية: 290 مل من محلول الملح، ثم تعرضت بعد ذلك إلى حالة من حالات التوتر، واعتبرت هذه المجموعة مجموعة التوتر وكان عددها ثمانية عشر فأرا. المجموعة الثالثة: 300 ميكروجرام لكل كيلو جرام من وزن الجسم من مادة البرنودوبريل وذلك قبل التعرض لحالات التوتر واعتبرت هذه المجموعة مجموعة التوتر المبلغة مسبقًا بالبرنودوبريل وكان عددها ثمانية عشر فأرا. وبعد ثلاثة أسابيع، قسمت المجموعة الثانية والثالثة إلى ثلاثة مجموعات فرعية متساوية يتكون كل منها من ستة فئران. تعرضت المجموعة الفرعية الأولى من كل إلى التوتر في صورة التعرض للجرارة والضوء، والمجموعة الفرعية الثانية إلى التوتر بالبرودة، أما المجموعة الفرعية الثالثة فنتج التوتر فيها بتطبيق الحركة والغموض في الماء. وقد تم أولاً دراسة تأثير أنواع هذا التوتر على النشاط السلوك، ثم دراسة رد الفعل الهرموني والعصبي، وكذا التغييرات الهيستوبولوجية في أنسجة الخصبة في هذه الفئران.

وقد بنت النتائج أن استخدام البرنودوبريل مسبقًا في مجموعة التوتر
بالتعرض للحرارة والضوء قد منع التغييرات المعنوية في هوموني الأدرنيكورتيكوتروبين والليستروبين والسلوك غير الاجتماعي، كما أدى إلى انخفاض معنوي؛ ولكن بدرجة بسيطة في الارتفاع الذي حدث في النوراينيفرن، ولكنه لم يمنع التغييرات المعنوية في الدوبامين والكورتيزول والحركات السلوكية التي أحدثها التوتر في المجموعة المقابلة وذلك بالمقارنة بالمجموعة الضابطة. كما أن استخدام هذا العقار مسبقاً أحدث زيادة معنوية في السلوك الاجتماعي ومادة السيروتونين إذا ما قورن بالمجموعة الضابطة. من ناحية أخرى، فإن العلاج المسبق بالبرندوريل في مجموعة التوتر بالتعرض للبرودة قد منع التغييرات المعنوية في النوراينيفرن والقدرة على التهديد التي أحدثها هذا النوع من التوتر في المجموعة المقابلة، ولكن أدى إلى ارتفاع معنوي في السلوك الاجتماعي بالمقارنة بالمجموعة الضابطة. أما في المجموعة العلاج مسبقاً بهذا العقار والتي تعرضت إلى تقييد الحركة والغمور في الماء، فقد أدى استخدام هذا العقار إلى منع حدوث التغييرات المعنوية في البرولاكتين والسلوك غير الاجتماعي، كما أدى أيضاً إلى انخفاض معنوي؛ ولكن بدرجة بسيطة في التغييرات في النوراينيفرن والدوبامين وهرمونات الأدرنيكورتيكوتروبين والكورتيزول والليستروبين وكذا القدرة على الدفاع، ولكنه حافظ على الزيادة المعنوية في السيروتونين التي حدثت في مجموعة التوتر المقابلة بالمقارنة بالمجموعة الضابطة. كما أن العلاج المسبق بالبرندوريل في هذه المجموعة قد أدى إلى زيادة معنوية في السلوك الاجتماعي وإلى انخفاض في الحركات السلوكية إذا ما قورن بالمجموعة الضابطة. ولم تظهر هذه الدراسة أي تغييرات هستوبولاجية في أنسجة الخصية سواءً في مجموعات التوتر أو في المجموعات المعالجة مسبقاً بالبرندوريل.