EFFECTS OF SKF-385 AND RESERPINE ON THE TISSUE CATECHOLAMINE CONTENT IN RABBITS

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The intravenous injection of 0.5 to 3.0 mg/kg of trans-2-phenylcyclopropylamine maleate (SKF-385) in the anesthetized dog produces the pressor effect which is prevented by the adrenolytic doses of tolazoline and dibenamine (1). However, the repetition of the doses of SKF-385 exerts the depressor effect which is totally abolished by the pretreatment with reserpine. The results indicate that the pressor effect relates with the release of the endogenous noradrenaline, while the depressor effect relates with the presence of the endogenous noradrenaline in the circulatory organs. The accumulation of the endogenous catecholamine after administration of monoamine oxidase (MAO) inhibitor has been shown in the central nervous system (2-4) and in the heart (5-7). On the other hand, the administration of iproniazid has been reported to produce no significant change of the brain noradrenaline in rabbit (8, 9) and of the heart noradrenaline in rabbit and mouse (9, 10). Goldberg and Shideman (11) have shown that the myocardial noradrenaline in cat decreases significantly in response to 2 to 30 mg/kg of SKF-385, while that in rat increases markedly.

Tachi, Nakatani and Fujiwara (12) have studied the effects of reserpine on the isolated atrial preparation of rabbit pretreated with the MAO inhibitors. The atrium of rabbit pretreated with beta-phenylisopropylamine or SKF-385, either of which alone produces the positive inotropic and chronotropic effects, has responded to reserpine with the depressor effect, while the atrium of rabbit pretreated with iproniazid has responded to reserpine with the augmenting effect. The results prompted to study the changes of catecholamine in the tissues including brain, heart, spleen and adrenal glands in the rabbit which received SKF-385 alone or reserpine after the pretreatment with SKF-385.

METHODS

Albino rabbits, weighing 1.5 to 2.5 kg and of both sexes, were used in the present experiments. The drugs used were trans-2-phenylcyclopropylamine maleate (SKF-385) and reserpine. The drugs were injected into the marginal vein of the ear. At 2, 4, 6, 8, 16, 24 and 32 hours after the injection of 5.0 mg/kg of SKF-385 the animals were sacrificed through bleeding by cutting both common carotid arteries.
In another series of experiments, reserpine in the dose of 1.0 mg/kg was injected intravenously, at 0, 2, 4, 6, 14, 24 and 30 hours after the administration of 5.0 mg/kg of SKF-385. Two hours thereafter, the animals were killed and the brain cortex, brain stem, atria, spleen and adrenal glands were extirpated and weighed for the determination of catecholamine in the tissues. The tissues were homogenized by adding 0.4 N-HClO₄. The homogenates of the tissues were prepared for the chemical assay of adrenaline and noradrenaline according to the method described by Higuchi (13). The content of noradrenaline in the brain cortex, brain stem and atria is expressed as μg/g wet tissue weight, while that of noradrenaline or adrenaline in the spleen and adrenal glands is expressed as total μg.

The contents of adrenaline or noradrenaline in the tissues of the intact rabbits are shown in Table 1. These values agree with those reported by Higuchi (13) except that in the brain stem where less level of the amine was detected.

### Table 1. The content of noradrenaline or adrenaline in the tissues of the intact rabbits.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Catecholamine</th>
<th>Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain cortex</td>
<td>NA</td>
<td>0.16 ± 0.01 μg/g</td>
</tr>
<tr>
<td>Brain stem</td>
<td>NA</td>
<td>0.24 ± 0.01 μg/g</td>
</tr>
<tr>
<td>Atria</td>
<td>NA</td>
<td>1.51 ± 0.07 μg/g</td>
</tr>
<tr>
<td>Spleen</td>
<td>NA</td>
<td>0.86 ± 0.10 μg</td>
</tr>
<tr>
<td>Adrenals</td>
<td>Ad</td>
<td>182 ± 5.9 μg</td>
</tr>
</tbody>
</table>

NA : Noradrenaline, Ad : Adrenaline.

### RESULTS

1. **Effects of SKF-385**

For the intravenous injection of SKF-385, the dose of 5.0 mg/kg which is enough to produce the consistent inhibition of the tissue MAO and the sympathomimetic effects was used. The intravenous injection of the dose of SKF-385 elicited a marked mydriasis, moderate degrees of tachypnoea and slight tachycardia. At 2, 4, 6, 8, 16, 24 and 32 hours after the injection the animals

### Table 2. The content of noradrenaline or adrenaline in the tissues of the intact rabbits received by 5.0 mg/kg of SKF-385.

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Brain cortex</th>
<th>Brain stem</th>
<th>Atria</th>
<th>Spleen</th>
<th>Adrenals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg/g % change</td>
<td>μg/g % change</td>
<td>μg/g % change</td>
<td>μg % change</td>
<td>μg % change</td>
</tr>
<tr>
<td>0</td>
<td>Mean 0.16</td>
<td>0.24</td>
<td>1.51</td>
<td>0.86</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S.E. 0.01</td>
<td>0.01</td>
<td>0.07</td>
<td>0.10</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>0.16</td>
<td>0.24</td>
<td>1.86</td>
<td>0.88</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.07</td>
<td>0.18</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.19</td>
<td>18.8</td>
<td>0.39</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.08</td>
<td>0.79</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.28</td>
<td>175.0</td>
<td>0.43</td>
<td>0.16</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.08</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.20</td>
<td>12.5</td>
<td>0.49</td>
<td>0.08</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.02</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.17</td>
<td>6.2</td>
<td>0.42</td>
<td>0.08</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.02</td>
<td>0.16</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.22</td>
<td>37.5</td>
<td>0.49</td>
<td>0.18</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.05</td>
<td>0.27</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0.17</td>
<td>6.2</td>
<td>0.35</td>
<td>0.11</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.04</td>
<td>0.10</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

% change: + increase, - decrease.
were sacrificed and the tissue contents of the amine were estimated. The results are shown in Table 2 and Figs. 1-5.

The intravenous injection of SKF-385 increased the level of noradrenaline in the brain cortex, brain stem and atria. The effect appeared slowly but progressively, and the highest level was obtained 6 or 8 hours after the injection. On the other hand, the amine level in the spleen was slightly increased or even reduced. In the adrenals a steady and moderate decrease of the level of adrenaline was observed, although the adrenaline level at 8 hours after the injection was considerably high. At 24 hours after the injection the level of noradrenaline in the brain cortex and brain stem was again high, while the level of noradrenaline in the atria showed a slight decrease. At 32 hours after the injection, the level of noradrenaline in the brain stem was still high, and that in the brain cortex and the spleen was in the physiological level, but that in the atria and adrenaline in the adrenals was much less than the physiological level.

2. Effects of reserpine in the rabbits pretreated with SKF-385

The effects of reserpine on the level of noradrenaline or adrenaline in the tissues of the rabbits injected 5.0 mg/kg of SKF-385 at 0, 2, 4, 6, 14, 24 and 30 hours previously were further studied. The amine levels were determined 2 hours after each injection of reserpine. The results are shown in Table 3 and Figs. 1-5. The pretreatment of the animal with SKF-385 modified the depleting effect of reserpine on the tissue amines. The effect of SKF-385 seemed to correlate with the time length from the injection of SKF-385 to that of reserpine.

### Table 3. The content of noradrenaline or adrenaline in the tissues of the SKF-385 pretreated rabbits 2 hours after injection of reserpine 1.0 mg/kg.

<table>
<thead>
<tr>
<th>Time after SKF-385</th>
<th>Brain cortex</th>
<th>Brain stem</th>
<th>Atria</th>
<th>Spleen</th>
<th>Adrenal glands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/g % change</td>
<td>µg/g % change</td>
<td>µg/g % change</td>
<td>µg % change</td>
<td>µg % change</td>
</tr>
<tr>
<td>Res. Mean S.E.</td>
<td>0.03</td>
<td>-91.2</td>
<td>0.08</td>
<td>-66.7</td>
<td>0.39</td>
</tr>
<tr>
<td>2</td>
<td>0.06</td>
<td>-62.4</td>
<td>0.18</td>
<td>-25.0</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>-7.3</td>
<td>0.25</td>
<td>+4.2</td>
<td>1.16</td>
</tr>
<tr>
<td>6</td>
<td>0.14</td>
<td>-12.5</td>
<td>0.29</td>
<td>+20.8</td>
<td>0.91</td>
</tr>
<tr>
<td>8</td>
<td>0.17</td>
<td>+6.3</td>
<td>0.35</td>
<td>+45.8</td>
<td>0.62</td>
</tr>
<tr>
<td>16</td>
<td>0.12</td>
<td>-25.0</td>
<td>0.21</td>
<td>-12.5</td>
<td>0.70</td>
</tr>
<tr>
<td>26</td>
<td>0.05</td>
<td>-62.4</td>
<td>0.15</td>
<td>-37.5</td>
<td>0.50</td>
</tr>
<tr>
<td>32</td>
<td>0.05</td>
<td>-68.7</td>
<td>0.12</td>
<td>-50.0</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Res.: The content of noradrenaline or adrenaline in the tissues of the intact rabbit 2 hours after injection of reserpine 1.0 mg/kg.

% change: + increase, decrease.
**Brain cortex:**

The decrease of the content of noradrenaline in the brain cortex 2 hours after the intravenous injection of 1.0 mg/kg of reserpine was 90% of the normal content. The simultaneous injection of SKF-385 and reserpine affected the noradrenaline depleting effect of reserpine on the brain cortex. Four hours after the injection of SKF-385 the noradrenaline depleting effect of reserpine was markedly reduced and the level of noradrenaline was near the physiological level. Six hours after the injection of SKF-385 when SKF-385 alone produced about 75% increase of the content of noradrenaline, the decrease of noradrenaline by reserpine in the brain cortex was only 12.5%. The results show that the injection of reserpine to the animal pretreated with SKF-385 depletes some amount of the tissue noradrenaline, but does not strikingly lower the tissue noradrenaline below the physiological level. However, 24 hours after the injection of SKF-385 when the content of noradrenaline in the brain cortex still showed a moderate increase (37.5%), reserpine caused a marked reduction of the tissue noradrenaline below the physiological level. Thirty-two hours after the injection of SKF-385, there was no further decrease.

**Brain stem:**

The pretreatment of the animal with SKF-385 modified the noradrenaline depleting effect of reserpine on the brain stem, but the pattern was somewhat different from that on the brain cortex. The decrease of the content of noradrenaline in the brain stem 2 hours after the injection of 1.0 mg/kg of reserpine was about 65% of the normal content.
content. The noradrenaline depleting effect of reserpine was markedly diminished to the decrease of about 25% at 2 hours after the combined injection of SKF-385. At 4, 6, and 8 hours after the injection of SKF-385 followed by reserpine, the content of brain stem noradrenaline was considerably low comparing with corresponding value of SKF-385 alone, but slightly higher than the physiological level. Eight hours after the injection of SKF-385 alone, there was a marked increase of the content of noradrenaline in the brain stem (104.2%) and the amine level was maintained until 24 hours after the injection. At 8 hours after the injection of SKF-385, reserpine produced a slight reduction of amine level, but the level was 45.8% higher than the physiological level. The injection of reserpine to the animal treated with SKF-385 24 hours previously produced a large reduction (37.8%) of the content of noradrenaline in the brain stem. Thirty-two hours after the injection of SKF-385 alone, there was still a marked increase (45.8%) of brain stem noradrenaline, but the injection of reserpine followed by SKF-385 produced a marked reduction, being similar to the effect of reserpine alone.

Atria:

The intravenous injection of 1.0 mg/kg of reserpine to the intact rabbit produced the reduction of the content of noradrenaline in the atria by about 75% of the normal content 2 hours later. Though 2 hours after the injection of SKF-385 the atrial noradrenaline showed a moderate increase by about 23%, 2 hours after the simultaneous injection of reserpine and SKF-385 the atrial noradrenaline showed about 70% decrease. Therefore, the depletion of the atrial noradrenaline by the simultaneous injection was sharply contrasted to the increase in the animal treated with SKF-385 alone. However, 4 and 6 hours after the injection of SKF-385 the decrease of the atrial noradrenaline due to reserpine was moderately depressed being 23.2% and 39.7%, respectively. The decrease of the atrial noradrenaline was more marked in the animal treated with SKF-385 8 hours previously. The content of the atrial noradrenaline 24 hours after the injection of SKF-385 alone showed still a slight increase above the physiological level. However, the injection of reserpine decreased the atrial noradrenaline of the animal treated with SKF-285 in almost the same extent as that of the intact animal. At 32 hours after the injection of SKF-385 alone, there was a slight decrease

FIG. 3. Noradrenaline content in the atria of rabbit after an injection of SKF-385 (5.0 mg/kg) and that of the SKF-385 pretreated rabbit 2 hours after an injection of reserpine (1.0 mg/kg).

Each point is an average of 3 to 5 animals. The vertical lines represent the range of the standard error of mean.

--- : physiological level.
(15.9%) of the atrial noradrenaline below the physiological level. At 32 hours after the injection of SKF-385, reserpine produced a marked reduction (55.6%) of the atrial noradrenaline.

**Spleen:**

Two hours after the injection of 1.0 mg/kg of reserpine the content of noradrenaline in the spleen was decreased by about 80% of the normal content. The simultaneous injection of the same dose of reserpine and SKF-385 did produce a marked depletion of the spleen noradrenaline as well as the injection of reserpine alone. However, the reduction of the spleen noradrenaline was much less in the animal pretreated with SKF-385 4 and 6 hours before than in the animal without treatment. The reduction of the spleen noradrenaline was the least in the animal treated with SKF-385 4 hours before. The increase of time interval between the injection of SKF-385 and reserpine intensified the decrease of the content of spleen noradrenaline. The decrease of the amine produced by reserpine in the animal pretreated with SKF-385 6 hours before was 41.9% and in the animal treated similarly 8 hours before was 61.6%. The content of spleen noradrenaline 24 hours after the injection of SKF-385 showed a decrease of 17.4% below the physiological level. The injection of reserpine to the animal treated with SKF-385 26 hours before produced a marked reduction (83.6%) of the content of the spleen noradrenaline. The content of noradrenaline in the spleen 32 hours after SKF-385 alone was not so far from the physiological level. However, the injection of reserpine decreased the spleen noradrenaline in the animal treated with SKF-385 in almost the same extent as that of the non-treated animal.

**Adrenal glands:**

The intravenous injection of 1.0 mg/kg of reserpine to the intact rabbit depleted the content of adrenaline in adrenal glands by about 40% of the normal content 2 hours later. The same dose of reserpine depleted 51.2% of the adrenaline content in the adrenal glands of the animal which received 5.0 mg/kg of SKF-385 2 hours before. However, the depletion of the amine content due to reserpine in the animal treated with SKF-385 4 hours before was only by 27.5% while SKF-385 alone produced 13.1%
reduction 4 hours later. The depletion of adrenaline in the adrenal glands due to reserpine 6 and 8 hours after the injection of SKF-385 was 50.6% and 37.6% of the normal content, respectively. There was a depletion of 33.5% of the normal content 24 hours after the injection of SKF-385 alone. At 26 hours after the injection of SKF-385, reserpine produced the depletion of 51.2% of the normal content of adrenaline in the adrenal glands. At 32 hours after the injection of SKF-385 alone the content of adrenaline in the adrenal glands showed the depletion of 51.7%. The depletion of adrenaline in the adrenal glands due to reserpine 32 hours after SKF-385 was 47.8%.

**DISCUSSION**

The intravenous administration of 5.0 mg/kg of SKF-385 in the intact rabbit exerted two types of change of the tissue catecholamine. The content of noradrenaline in the brain cortex, brain stem and atria showed a steady increase, while the content of noradrenaline in the spleen and adrenaline in the adrenal glands showed a variable effect or rather a decrease. The increased level of noradrenaline in the brain cortex was in peak 4 to 8 hours after the injection and thereafter declined slowly. But, some increase was still observed 24 hours after the injection. The level of noradrenaline in the brain stem increased progressively from 4 hours after the injection and reached a plateau at 8 hours. The average increase from 8 hours to 24 hours after the injection was almost 100%. At 32 hours a considerable increase was still observed. The increase of the level of noradrenaline in the atria started from 2 hours after the injection and lasted until 24 hours. The peak effect of about 50% increase was observed at 6 hours. Though the content of adrenaline in the adrenal glands and of noradrenaline in the spleen showed a variable value, a slight but progressive decrease was common for both tissues. The peak decrease was observed at 16 hours in the spleen and at 32 hours in the adrenal glands. Goldberg and Shideman (11) have explained the difference of the change in myocardial noradrenaline due to SKF-385 between cats and rats, from the difference of the metabolic pathway of catecholamine in both species. They have suggested that catecholamine in rats is inactivated mainly by MAO, while that in cats is by COMT (catechol-O-methyltransferase). The higher activity of MAO than COMT
in the brain and heart shown by Crout et al. (14) may relate with the increase in the
noradrenaline content in those tissues after the injection of SKF-385. In the spleen
and adrenal glands the central sympathetic stimulation produced by SKF-385 may lead
to the release of the endogenous amine with subsequent decrease of catecholamine level.
However, direct evidence to support the assumption is lacking.

At 0, 2, 4, 6, 14, 24 and 30 hours after the administration of 5.0 mg/kg of SKF-385,
reserpine in the dose of 1.0 mg/kg of SKF-385, reserpine in the dose of 1.0 mg/kg was
injected intravenously. Two hours after reserpine the animal were killed for the deter-
mination of catecholamine in the tissues. The simultaneous injection of SKF-385 and
reserpine produced almost the same degree of the catecholamine depletion as the in-
jection of reserpine alone. However, the injection of reserpine 2 hours or more after
the injection of SKF-385 produced less depletion of tissue catecholamine than depletion
after reserpine alone. The time course of the depletion differed considerably according
to the tissues. In the brain cortex the least depletion due to reserpine observed 8 hours
after SKF-385. The depleting effect of reserpine on the noradrenaline content was
prevented a little for 16 hours following the pretreatment of SKF-385 and thereafter
was prevented no more. The time course of the depletion of brain stem noradrenaline
due to reserpine in the animal pretreated with SKF-385 was almost the same as that
of the brain cortex noradrenaline. The content of the atrial noradrenaline was reduced
by reserpine in lesser degree after the treatment with SKF-385, but the effect of treat-
ment was marked only for 3 to 6 hours after SKF-385. The administration of reser-
pine in the rabbit pretreated with SKF-385 produced a variable depletion of noradre-
naline in the spleen and of adrenaline in the adrenal glands, but the depletion was
generally less in the animal pretreated with SKF-385 than in the intact animal.

From the results described above, it is concluded that the pretreatment with SKF-
385 protects the depletion of tissue catecholamine due to reserpine and the protection
is most marked and sustained in the brain. Though no increase above the physiolo-
gical level of catecholamine in the tissues was observed, the marked prevention by
SKF-385 of the reserpine depletion of the brain noradrenaline may be interpreted from
higher activity of brain MAO than the activity of the brain COMT suggested by
Goldberg and Shideman (11). However, the less preventing effect of SKF-385 in the atrial
noradrenaline could not be explained from the assumption, because of higher activity
of MAO than COMT in the heart. In this regards, it is suggestive that Carlsson et al.
(15) have shown the sensitive vulnerability of the heart noradrenaline to reserpine.
Pepeu et al. (16) have shown that the isolated guinea-pig's heart responded to the com-
bined administration of reserpine and iproniazid with the accumulation of the heart
noradrenaline, where the combined administration of reserpine and beta-phenylisoprop-
pyridazine depletes the heart noradrenaline as almost the same degree as reserpine
alone. No reserpine reversal on the level of the tissue catecholamine may relate with
the specific pharmacological effect of SKF-385 or alternatively with the species difference
between the guinea-pig and rabbit.
SKF-385 ON CATECHOLAMINE

SUMMARY

Concentrations of noradrenaline in the brain cortex, brain stem, atria and spleen and adrenaline in the adrenal glands in rabbits were determined at various time intervals after the administration of SKF-385 and at 2 hours after the administration of reserpine pretreated with SKF-385.

The administration of SKF-385 alone in the rabbit exerted two types of change of tissue catecholamine. The content of noradrenaline in the brain cortex, brain stem and atria showed a steady increase. The largest increase was obtained in the brain stem. The content of noradrenaline in the spleen and adrenaline in the adrenal glands showed a variable effect or rather decrease.

The administration of reserpine in the rabbit pretreated with SKF-385 produced a variable change of catecholamine in the rabbit tissues. The pretreatment with SKF-385 protected from the depletion of tissue catecholamines due to reserpine and the protection was the longest-lasting in the brain.

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