CARDIOVASCULAR STUDIES OF 5-(3-TERT-BUTYLAMINO-2-HYDROXY) PROPOXY-3,4-DIHYDROCARBOSTYRIL HYDROCHLORIDE (OPC-1085), A NEW POTENT \(\beta\)-ADRENERGIC BLOCKING AGENT

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Abstract—The \(\beta\)-adrenergic blocking and hemodynamic properties of 5-(3-tert-butylamino-2-hydroxy) propoxy-3,4-dihydrocarbostyril hydrochloride (OPC-1085) were investigated and compared with those of propranolol and pindolol on myocardial contractile force, heart rate and arterial blood pressure in pentobarbital anesthetized dogs. OPC-1085 showed almost the same \(\beta\)-adrenergic blocking potency as pindolol, but was approximately 30 and 20 times stronger than propranolol against isoproterenol and cardiac nerve stimulation, respectively. Slight negative inotropic and chronotropic responses were observed with OPC-1085 in effective blocking doses from 1 to 10 \(\mu\)g/kg but were converted to positive ones in doses from 30 to 3000 \(\mu\)g/kg in non-reserpinized dogs. In reserpinized dogs, both OPC-1085 and pindolol induced only positive responses. These responses were more evident with OPC-1085.

Since the discovery of dichloroisoproterenol by Powell and Slater (1), numerous \(\beta\)-adrenergic blocking agents have been synthetized. Among them, propranolol was first introduced in clinical medicine (2) and is now widely prescribed for ischemic heart diseases, hyperkinetic syndrome, cardiac arrhythmias, hypertension etc, although propranolol has a well-known myocardial depressant effect (3, 4). Pindolol (5, 6, 7) and Kö 1366 (8, 9, 10) have been reported as more potent \(\beta\)-adrenergic blocking agents which have practically no depressant effect.

Nakagawa et al. (11) described antagonistic action of a series of dihydrocarbostyril derivatives against isoproterenol, among which 5-(3-tert-butylamino-2-hydroxy) propoxy-3, 4-dihydrocarbostyril hydrochloride (OPC-1085) was the most promising compound because of its potent effect with duration of action as long as that of pindolol. In this study, we investigated the \(\beta\)-adrenergic blocking properties of OPC-1085 and compared them with those of propranolol and pindolol on cardiovascular responses of the dog.

MATERIALS AND METHODS

Fifty adult mongrel dogs of both sexes, weighing from 10 to 20 kg, were anesthetized with sodium pentobarbital, 30 mg/kg i.v. In forty-five dogs, both vagus nerves had been cut at the mid-cervical level. Intubation was carried out and the tracheal tube was attached to a respirator. The chest was opened by the mid-line incision and the heart was suspended in the pericardial cradle. The contractile force of the myocardium was mea-
sured by means of a Walton-Brodie strain-gauge arch sutured on to the right ventricle. Arterial blood pressure at the femoral artery was recorded by an electromanometer and the heart rate by a cardiotachograph which was triggered by R waves of ECG (Lead II). In fifteen dogs, the right cardiac nerve was stimulated for 30 sec with square-wave pulses; 1 msec duration, 10 Hz and supramaximal voltage (6–8 V), and in five dogs, the right vagus nerve for 20 sec with the following stimulus; 1 msec duration, 2, 5 or 10 Hz and supramaximal voltage (2–5 V).

Drugs used in these experiments were 1-isoproterenol hydrochloride (Nikken Kagaku), dl-epinephrine hydrochloride, dl-norepinephrine hydrochloride (Sankyo) and calcium chloride as agonists, while propranolol hydrochloride (ICI), pindolol (LB-46, Sankyo) and 5-(3-tert-butylamino-2-hydroxy) propoxy-3, 4-dihydrocarbostyril hydrochloride (OPC-1085, Otsuka) as antagonists and reserpine (Ciba). All drugs were dissolved in 0.9% saline except for pindolol which was dissolved in 0.9% saline with equimolar maleic acid. The injected volume was 0.1 ml/kg which was administered through a catheter into the femoral vein in a period of 1 min. The i.v. administration of agonists and nerve stimulation were performed 10 min after each injection of β-adrenergic blocking agent in cumulative doses of 1, 3, 10, 30, 100, 300 and 1000 µg/kg at 20 min-intervals. The ED50 for blocking cardiovascular responses to isoproterenol and right cardiac nerve stimulation was estimated from straight lines obtained by plotting the inhibition in percent against a logarithmic dose.

Thirty dogs were used to investigate the direct actions of OPC-1085, pindolol and propranolol on the cardiovascular system, fifteen dogs of which were pretreated with reserpine for 2 days, 0.5 mg/kg followed by 1 mg/kg s.c. Myocardial contractile force, heart rate and arterial blood pressure were measured in a similar way for reserpinized dogs but with less anesthetic doses of sodium pentobarbital (20 mg/kg i.v.). These β-adrenergic blocking agents were injected intravenously in cumulative doses from 1 µg/kg to 10 mg/kg at 5 min-intervals and the cardiovascular parameters were determined 5 min after administration of each dose.

RESULTS

The ED50 of β-adrenergic blocking actions of OPC-1085, pindolol and propranolol against isoproterenol

Isoproterenol, 0.3 µg/kg i.v., produced positive inotropic and chronotropic actions and a marked hypotension which lasted from 2 to 8 min (Fig. 1). These cardiovascular responses to isoproterenol as an agonist were dose-dependently depressed by OPC-1085, pindolol and propranolol in a dose range from 1 to 30 µg/kg for the former two and from 10 to 300 µg/kg i.v. for the latter one. OPC-1085, 30 µg/kg, completely inhibited these responses to isoproterenol but not completely with 30 µg/kg of pindolol or 300 µg/kg of propranolol (Fig. 1). The ED50 of OPC-1085, pindolol and propranolol as antagonists is tabulated in Table 1 and was found to be almost the same between OPC-1085 and pindolol, i.e., approximately 30 times more potent than propranolol.
The ED50 of antagonistic actions of OPC-1085, pindolol (LB-46) and propranolol as related to antagonization of the cardiovascular responses to isoproterenol, 0.3 μg/kg i.v., in vagotomized dogs. Each value represents mean ± SE.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Contractile force</th>
<th>Heart rate</th>
<th>Blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC-1085</td>
<td>3.1 ± 0.25</td>
<td>2.3 ± 0.34</td>
<td>1.8 ± 0.30</td>
</tr>
<tr>
<td>LB-46</td>
<td>4.6 ± 0.45</td>
<td>3.4 ± 0.77</td>
<td>2.8 ± 0.59</td>
</tr>
<tr>
<td>Propranolol</td>
<td>112.7 ± 3.05</td>
<td>71.7 ± 3.41</td>
<td>71.7 ± 4.35</td>
</tr>
</tbody>
</table>

The ED50 of antagonistic actions of OPC-1085, pindolol and propranolol against right cardiac nerve stimulation

The positive inotropic and chronotropic actions elicited by stimulation of the right cardiac nerve were depressed by OPC-1085, pindolol and propranolol in a dose range from
I to 100 μg/kg for the former two and 10 to 1000 μg/kg i.v. for the latter one. The dose response curves of inhibitory effects of OPC-1085, pindolol and propranolol are parallel as shown in Fig. 2. The ED50 of these drugs is tabulated in Table 2. OPC-1085 and pindolol have almost the same potency, that is approximately 20 times more potent than propranolol.

**TABLE 2.** ED50 of OPC-1085, pindolol (LB-46) and propranolol as related to antagonization of the cardiovascular responses to electrical stimulation (10 Hz, 1 msec, 6-8 V, 30 sec) of the right cardiac nerve in vagotomized dogs. Each value represents mean ± SE.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Contractile force</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC-1085</td>
<td>5.2 ± 0.93</td>
<td>6.1 ± 0.79</td>
</tr>
<tr>
<td>LB-46</td>
<td>7.3 ± 1.47</td>
<td>8.3 ± 0.81</td>
</tr>
<tr>
<td>Propranolol</td>
<td>112.8 ± 32.74</td>
<td>138.0 ± 36.86</td>
</tr>
</tbody>
</table>

1 to 100 μg/kg for the former two and 10 to 1000 μg/kg i.v. for the latter one. The dose response curves of inhibitory effects of OPC-1085, pindolol and propranolol are parallel as shown in Fig. 2. The ED50 of these drugs is tabulated in Table 2. OPC-1085 and pindolol have almost the same potency, that is approximately 20 times more potent than propranolol.

**Inhibitory effects of OPC-1085 on cardiovascular responses to epinephrine and norepinephrine**

The positive inotropic and chronotropic actions induced by 1 μg/kg i.v. of epinephrine or norepinephrine were inhibited by OPC-1085 in a dose range from 1 to 30 μg/kg i.v.
However, the hypertension induced by epinephrine was enhanced by OPC-1085 but that by norepinephrine remained unchanged (Fig. 3).

**Absence of inhibitory effect of OPC-1085 on CaCl₂ and vagal stimulation**

CaCl₂, 15 mg/kg i.v., induced a marked positive inotropic response with a slight positive chronotropic response lasting from 4 to 6 min. The positive inotropic action of CaCl₂ was not inhibited by OPC-1085 in a dose range from 0.1 to 3 mg/kg i.v. (Fig. 4).

![Graph showing effects of OPC-1085 on cardiac contractile force, heart rate, and arterial blood pressure](image-url)

*Fig. 3. Effect of OPC-1085 on responses of cardiac contractile force (CF), heart rate (HR) and arterial blood pressure (BP) to epinephrine (EP), 1 μg/kg i.v., and norepinephrine (NE), 1 μg/kg i.v., in vagotomized dogs.*

![Graph showing absence of blocking effect of OPC-1085 on CaCl₂ response](image-url)

*Fig. 4. Absence of blocking effect of OPC-1085 on responses of cardiac contractile force (CF), heart rate (HR) and arterial blood pressure (BP) to calcium chloride (CaCl₂), 15 mg/kg i.v., in vagotomized dogs.*
Furthermore, a negative chronotropic response to right vagal nerve stimulation was not modified by OPC-1085 in a dose range from 1 to 5 mg/kg i.v. (Fig. 5).

Effects of OPC-1085, pindolol and propranolol on the myocardial contractile force and arterial blood pressure in non-reserpinized and reserpinized dogs

In fifteen non-treated dogs, control values of heart rate and mean systemic blood pressure were 152 ± 4.1 beats/min (mean ± SE) and 127 ± 4.4 mm Hg (mean ± SE), re-

Fig. 5. Absence of blocking effect of 1-5 mg/kg of OPC-1085 on responses of arterial blood pressure (BP) and heart rate (HR) to vagus nerve stimulation (ES).

Fig. 6. Changes in cardiac contractile force (CF), heart rate (HR) and arterial blood pressure (BP) after cumulative dose application of OPC-1085, pindolol (LB-46) and propranolol in vagotomized dogs (n=5, mean ± SE).

Fig. 7. Changes in cardiac contractile force (CF) and heart rate (HR) after cumulative dose application of OPC-1085, pindolol (LB-46) and propranolol in reserpinized and vagotomized dogs (n=5, mean ± SE).
A NEW \( \beta \)-BLOCKER; OPC-1085

respectively. Negative chronotropic responses were induced dose-relatively by propranolol. The negative chronotropic responses to OPC-1085 and pindolol were almost the same within a range from 1 to 30 \( \mu \)g/kg i.v. but were converted to positive ones with a higher dose of OPC-1085 or pindolol. The highest dose of pindolol used in this study, however, induced a repeat of the negative chronotropic response. Hypotensive responses to OPC-1085, pindolol and propranolol were observed at 300 \( \mu \)g/kg i.v. but the most significant hypotension was induced by propranolol, less by pindolol and least by OPC-1085. Inotropic responses to OPC-1085 and pindolol were negative within a range from 1 to 10 and from 1 to 30 \( \mu \)g/kg i.v., respectively but converted to positive ones which reached the maximum at 1 mg/kg i.v. OPC-1085 never induced a negative inotropic response even with the highest dose of 10 mg/kg i.v. used in this study however, pindolol induced a negative response when the dose was over 1 mg/kg i.v. Propranolol dose-relatedly induced a negative response (Fig. 6).

In fifteen reserpinized dogs, the control values of heart rate and mean systemic blood pressure were 100 \( \pm \) 3.0 beats/min (mean \( \pm \) SE) and 75 \( \pm \) 2.5 mmHg (mean \( \pm \) SE), respectively. These values were obviously lower than those observed in non-treated dogs. OPC-1085 and pindolol induced positive inotropic and chronotropic responses even with the smallest dose of 1 \( \mu \)g/kg i.v. used in this study, but OPC-1085 was more potent than pindolol. On the other hand, propranolol produced negative inotropic and chronotropic effects with 30 and 300 \( \mu \)g/kg i.v., respectively. These effects were always significantly weaker in reserpinized dogs (Fig. 7).

DISCUSSION

The present authors made an attempt to investigate the difference in \( \beta \)-adrenergic blocking properties among OPC-1085, propranolol and pindolol on cardiovascular effects in dogs. OPC-1085 dose-dependently inhibited the positive inotropic, positive chronotropic and hypotensive actions induced by isoproterenol i.v. The blocking potency of OPC-1085 was almost the same as that of pindolol which was approximately 30 times stronger than that of propranolol. OPC-1085 and pindolol inhibited the hypotensive response to isoproterenol more than the positive chronotropic and inotropic ones. Previously three types of \( \beta \)-adrenergic blocking agents, i.e. \( \beta_1 \), \( \beta_2 \) and \( \beta_1 \cdot \beta_2 \), have been pointed out, and \( \beta_1 \) was proposed for the cardiac function and \( \beta_2 \) receptors for responses of the vascular and tracheal smooth muscles and others (12, 13, 14). As a matter of fact, practolol, a selective \( \beta_1 \) blocking agent was introduced by Dunlop and Shanks (15) and isopropyl-methoxamine or buthoxamine, each selective \( \beta_2 \) blocking agent by Levy in 1964 (16) and 1966 (17). OPC-1085 exerted its effect on both \( \beta_1 \) and \( \beta_2 \) receptors as did propranolol and pindolol. OPC-1085, propranolol and pindolol also inhibited the positive inotropic and chronotropic responses to cardiac nerve stimulation. The dose response curves of inhibitory effect of OPC-1085, propranolol and pindolol against cardiac nerve stimulation were parallel, and the blocking potencies of OPC-1085 and pindolol were approximately 20 times more potent than that of propranolol. The parallelism in inhibitory
dose response curves of OPC-1085, pindolol and propranolol suggest that OPC-1085 has similar \( \beta \)-adrenergic blocking properties to pindolol and propranolol. Cheymol et al. (9) reported that Kö 1366, one of \( \beta \)-adrenergic blocking agents, depressed the effects of nerve stimulation more than the effects of isoproterenol, and thus they suggested an inhibition of norepinephrine release by Kö 1366. The present study shows no such effect of OPC-1085.

OPC-1085 inhibited the positive inotropic and chronotropic responses to epinephrine and norepinephrine. It potentiated the pressor response to epinephrine, but not to norepinephrine. In 1966, Shanks (3) reported that propranolol blocked the vasodilator response to epinephrine and potentiated the pressor response to epinephrine. In this study, it appeared that the potentiation of the pressor response to epinephrine by OPC-1085 was due to a block of the peripheral vasodilator action of epinephrine without affecting its vasoconstrictor action such as was seen with propranolol.

Furthermore in the present experiments, OPC-1085 was proven to be a specific \( \beta \)-adrenergic blocking agent as it has no effect on the positive inotropic response induced by \( \text{CaCl}_2 \), bradycardia to vagal nerve stimulation and pressor response to norepinephrine.

OPC-1085 and pindolol showed definite positive inotropic and chronotropic responses in reserpinized dogs, while slight negative ones in non-treated animals in a range from 1 to 30 \( \mu \text{g/kg} \) i.v. which is the effective dose as a \( \beta \)-adrenergic blocking agent. These observations indicate the existence of a prevailing sympathetic tone in dogs anesthetized with sodium pentobarbital. These positive inotropic and chronotropic effects were more prominent in reserpinized dogs. Thus we conclude that OPC-1085 has a sympathomimetic action which is well known with alprenolol (18, 19), Kö 1366 (10) and dichloroisoproterenol (20). Hashimoto et al. (21) and Ohkuda et al. (22) have observed that an intraarterial injection of a smaller dose of dichloroisoproterenol, pindolol, alprenolol or practolol produced a positive chronotropic response in the in situ sino-atrial node preparation which is the same as was seen with Kö 1366, pindolol or practolol in the isolated blood-perfused one (23). OPC-1085 induced a more prominent sympathomimetic effect with cumulative administration of increasing doses than did pindolol.

Negative inotropic and chronotropic responses were never induced with any dose of OPC-1085 but such responses were evident with pindolol in very large doses. The negative inotropic and chronotropic responses to pindolol were significantly weaker than those of propranolol which is in agreement with previous reports (5, 6, 7).

From these studies, it is concluded that OPC-1085 is one of the most potent \( \beta \)-adrenergic blocking agents devoid of negative inotropic and chronotropic actions with sympathomimetic effects. Furthermore, it was demonstrated that OPC-1085 has a weaker hypotensive action than that of pindolol or propranolol.

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REFERENCES