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# TISSUE SELECTIVITY OF PROPRANOLOL DERIVATIVES *IN VIVO*

## A CONFIRMATION OF *IN VITRO* FINDINGS

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**Abstract**—The β-adrenergic antagonist activities of a *p*-toluidide and a *p*-trifluoromethylanilide derivative of propranolol were tested in intact rats to determine whether the unusual *in vitro* profiles on myocardium and adipose tissue were found *in vivo*. The relative potencies of *p*-toluidide derivatives studied in pithed rats were 1.3 for the rate of change in left ventricular pressure with respect to time (dP/dt) and 4.6 for heart rate (HR) compared to propranolol. The values for *p*-trifluoromethylanilide were 2.5 (dP/dt) and 4.6 (HR). The *SR*-isomer of the *p*-toluidide derivative was 81 times more potent than propranolol in inhibiting effects on dP/dt and 27 times more effective than propranolol on HR, whereas the *SS*-isomer was 0.17 times (dP/dt) and 0.16 times (HR) as potent as the parent compound. In fasted rats, infusion of isoproterenol resulted in an increase of 10.6 ± 3.1 mg/dl in plasma non-esterified fatty acid (NEFA) and an increase of 46 ± 9 mg/dl in glucose. Unlike propranolol, neither the *p*-toluidide nor the *p*-trifluoromethylanilide derivative blocked the increase in plasma NEFA, although they both blocked the increase in plasma glucose. It appeared that the *p*-toluidide and *p*-trifluoromethylanilide derivatives of propranolol were more selective for the $\beta_1$-adrenergic receptors on the heart as opposed to the $\beta_1$-like adrenoceptors on adipose tissue. These findings were qualitatively and generally quantitatively in agreement with our previous findings *in vitro*. Therefore, the *in vitro* data may be useful in predicting atypical and tissue selective *in vivo* effects of these types of compounds.

β-Adrenergic agonists and histamine can be attached covalently to complex, but pharmacologically inert, carriers without loss of some of the pharmacologic activity of the progenitor. It has been demonstrated that a series of congener derivatives and conjugates of norepinephrine had unusual pharmacologic effects [1–4]. Several of the derivatives were more potent than their progenitor, and some had unexpected receptor, tissue and effect selectivity [5, 6]. A series of derivatives of propranolol and practolol were synthesized that were chemically analogous to the previously reported norepinephrine derivatives [1–4]. The congener derivatives of propranolol were more tissue selective than the progenitor. For example, the *p*-trifluoromethylanilide derivative had 400-fold more affinity for $\beta_1$-adrenergic receptors on heart as opposed to $\beta_1$-like receptors of adipose tissue [7]. In this paper we report *in vivo* effects of the *p*-toluidide and *p*-trifluoromethylanilide derivatives of propranolol and the *SR* and *SS* enantiomers of the *p*-toluidide derivative.

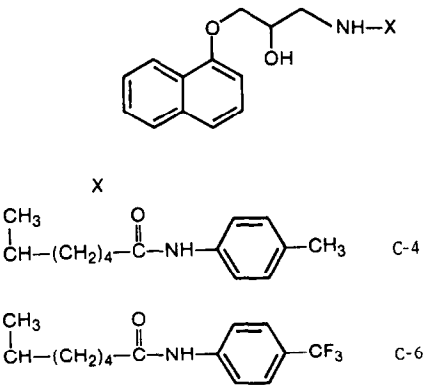


Fig. 1. Structures of the propranolol derivatives designated C-4 and C-6.

## METHODS

*Pharmacologic agents.* The details of the synthesis of congener derivatives have been described [7]. The *p*-toluidide (C-4)§ and *p*-trifluoromethylanilide (C-6) derivatives of propranolol (Fig. 1), the *SR*-isomer of C-4 (C-51) and the *SS*-isomer of C-4 (C-52), *dl*-isoproterenol sulfate dihydrate (Aldrich, Milwaukee, WI) and *dl*-propranolol HCl (Sigma Chemical Co., St. Louis, MO) were used. The two stereoisomers were synthesized separately by epoxide ring opening of (*S*)-3-naphthyloxy-1,2-epoxy-

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§ Abbreviations: C-4, *p*-toluidide derivative of propranolol; C-6, *p*-trifluoromethylanilide derivative of propranolol; C-51, *SR*-isomer of C-4; C-52, *SS*-isomer of C-4; BP, blood pressure; LVP, left ventricular pressure; HR, heart rate; dP/dt, the rate of change in LVP with respect to time; and NEFA, non-esterified fatty acid.

propane with either the (*R*)- or (*S*)-6-aminoheptonoic acid toluidide. The chiral aryloxy-epoxypropane was synthesized utilizing a product of the Sharpless asymmetric epoxidation method. Compound purity was determined using several criteria. All melting points were sharp, and the microchemical analysis of products showed the expected values. TLC in several solvent systems showed a single homogeneous spot. The 360 MHz proton NMR showed all expected resonances and no extraneous bands. Where a mixture of diastereomers gave a quartet for the asymmetric methyl group, a doublet was observed for the diastereomerically clean material (limit of the NMR more than 2%). Reverse phase HPLC showed a single peak. This technique could not be used to establish optical purity since each pair of diastereomers eluted at the same place under all the elution conditions attempted. Details of the procedure will appear in a future publication.

Enantiomeric purity was determined by synthesizing the Mosher amide of both the chiral amino-toluidide and the amino-trifluoromethyl followed by observation of the proton NMR [8]. A mixture of amino-amide enantiomers results in a mixture of Mosher amide diastereomers which is readily visible in the NMR. Only enantiomerically clean material was observed for all compounds tested.

The solutions were prepared in saline such that a volume of 0.1 ml/100 g body weight was administered. All derivatives of propranolol were prepared in methanol first and then in saline in the same volume as described above. Approximately 0.3 ml of saline was used to wash in the drugs after each intravenous injection.

*Effects on cardiac contractility and heart rate.* Male Sprague–Dawley rats (Bantin-Kingman, Inc., Fremont, CA) weighing 270–330 g were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). The trachea was cannulated for artificial respiration (1.5 ml/100 g body weight, 50 strokes/min) with a Harvard respirator. The left femoral vein and artery were cannulated with PE-50 tubing filled with heparinized saline. A Statham pressure transducer (P23Db) was connected to the arterial cannula for measurement of the blood pressure (BP). The femoral vein was utilized for administration of drugs. A PE-50 tubing was placed into the left ventricle via the right carotid artery and secured in place for measurement of the left ventricular pressure (LVP), from which dP/dt and heart rate (HR) were derived.

The rats were pithed through the left orbit with a stainless steel rod as described previously [9]. Prior to pithing, the rats were pretreated with the following compounds: atropine (0.5 mg/kg, i.v., to prevent vagal parasympathetic stimulation), chlorisondamine (2.5 mg/kg, i.p., to eliminate untoward reactions to massive release of catecholamines) and gallamine triethodide (10 mg/kg, i.v., to relax skeletal muscles). Body temperature was maintained with an Aquamatic K-module pad.

Isoproterenol (0.004, 0.04, 0.4 and 4.0 μmol/kg) was administered intravenously in an ascending dose manner to rats at 10-min intervals between doses. When test compounds were used, they were administered (1 μmol/kg) 20 min later; the isoproterenol dose response was then repeated. BP, LVP, dP/dt and HR were recorded continuously on a Beckman R511A Dynograph.

*Effects on plasma NEFA and glucose.* Male Sprague–Dawley rats (Batin-Kingman) weighing 180–210 g were fasted overnight, and anesthetized with pentobarbital sodium (60 mg/kg, i.p.). The left femoral vein and right carotid artery were cannulated with PE-50 tubing filled with normal saline. The separate cannulae were used for administration of drugs and collection of samples. Twenty minutes after administration of test compounds, the rats were infused with isoproterenol (8 μmol/kg/min) at 0.2 ml/min or normal saline (0.2 ml/min) for 15 min. Before and 20 min after the infusion of isoproterenol or saline, carotid blood samples (0.5 ml) were collected into centrifuge tubes containing 55 μl of 3.8% sodium citrate and placed on ice.

Plasma NEFA was determined enzymatically using a colorimetric method [10] provided as a kit from Wako Pure Chemicals (NEFA-C, Dallas, TX). The plasma glucose levels were measured using an enzymatic colorimetric procedure developed by Keston and Brandt [11] and modified by Raabo and Terkildsen [12]. The reagents were purchased from the Sigma Chemical Co.

*Statistical analysis.* Data are expressed as mean ± SEM and analyzed using Student's two-tailed *t*-test for unpaired observations. Values were considered to be statistically significant at $P < 0.05$.

## RESULTS

*Effects on cardiac contractility and heart rate.* Intravenous administration of isoproterenol (0.004, 0.04, 0.4 and 4.0 μmol/kg, i.v.) to the pithed rat induced dose-dependent increases in dP/dt and heart rate (HR). At 4 μmol/kg i.v., isoproterenol induced a 70% increase in dP/dt and a 40% increase in HR. The initial values for dP/dt and HR were 4525 ± 605 mm Hg/sec (N = 4) and 322 ± 30 beats/min (N = 4) respectively. Repeated challenges of isoproterenol produced almost identical dose-responses, indicating that the interval between doses was sufficient to prevent tachyphylaxis to the agonist. Propranolol (1 μmol/kg), C-4 (1 μmol/kg), and C-6 (1 μmol/kg) shifted the dose–response curves to the right in a parallel fashion. Propranolol, C-4 and C-6 altered the $ED_{50}$ values of isoproterenol on dP/dt to 0.72, 0.96 and 1.8 μmol/kg, respectively, and 1.48, 6.84 and 6.84 μmol/kg for HR (Table 1). Thus, the C-4 and C-6 compounds were 1.3 and 2.5 times more potent than propranolol in blocking the response of isoproterenol-induced changes in dP/dt, while both compounds were 4.6 times more potent than propranolol in blocking the response in HR. The relative effects of the derivatives on HR were four times greater than on dP/dt. Although the two cardiac functions are concomitant during β-adrenergic stimulation, they are separate and differentiable by these blockers.

As shown in Table 1, the *SR*-isomer was more potent than the racemic mixture in its effects on both dP/dt and HR. The *SR*-isomer was 81 times more potent than propranolol on dP/dt and 27 times more potent than propranolol on HR. In contrast, the *SS*-

Table 1. *In vivo* pharmacologic activity of derivatives of propranolol

| Compound | Dose (μmol/kg) | dP/dt $ED_{50}$ of Isoproterenol* (μmol/kg) | dP/dt Relative potency | HR $ED_{50}$ of Isoproterenol* (μmol/kg) | HR Relative potency |
|---|---|---|---|---|---|
| Propranolol | 1 | 0.72 | 1.00 | 1.48 | 1.00 |
| C-4† | 1 | 0.96 | 1.33 | 6.84 | 4.62 |
| C-6 | 1 | 1.8 | 2.50 | 6.84 | 4.62 |
| C-51‡ | 1 | 58.4 | 81.11 | 39.9 | 27.00 |
| C-52§ | 1 | 0.12 | 0.17 | 0.24 | 0.16 |

* Dose of isoproterenol required to induce 50% change in dP/dt or HR.
† Racemic mixture.
‡ *SR*-isomer of C-4.
§ *SS*-isomer of C-4.

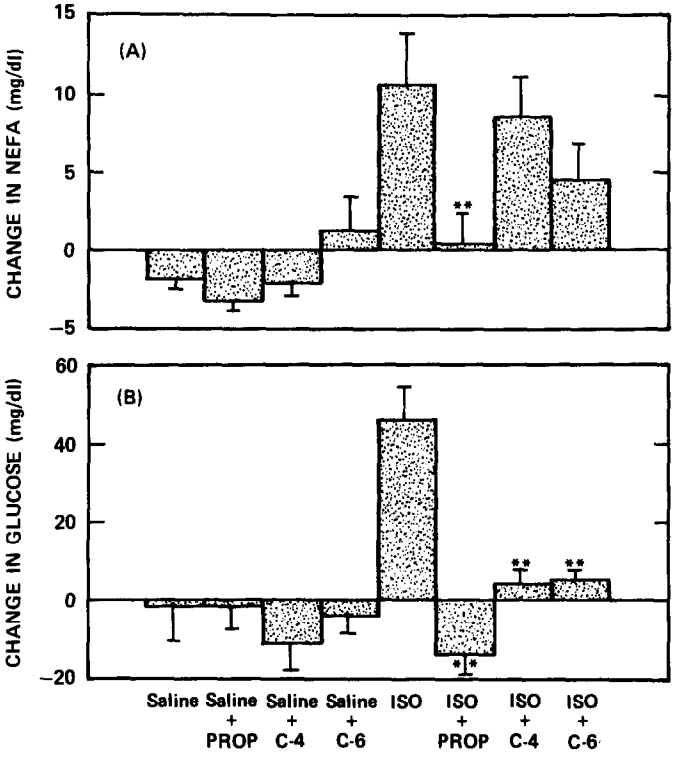


Fig. 2. Inhibitory effects of derivatives of propranolol on isoproterenol-induced changes in plasma NEFA (A) and glucose (B). Key: (**) $P < 0.01$ compared to isoproterenol-induced changes.

isomer was 6 times less potent than propranolol in blocking isoproterenol dP/dt and HR responses.

*Effects on plasma NEFA and glucose.* The control values of NEFA and glucose in fasted rats were 17.7 ± 0.8 and 83 ± 8 mg/dl respectively. These values did not change significantly during infusion of saline nor during or after the administration of any of the test compounds given alone.

Infusion of isoproterenol generated increases in plasma NEFA and glucose (Fig. 2). The increments after 20 min of infusion of isoproterenol were 10.6 ± 3.1 mg/dl in NEFA ($P < 0.01$) and 46 ± 9 mg/dl in glucose ($P < 0.01$). When 10 μmol/kg of propranolol, C-4 or C-6 was given followed by an infusion of isoproterenol, the increments of NEFA were 0.3 ± 1.7, 8.8 ± 2.5 and 4.4 ± 2.3 mg/dl and the increments in glucose were −4 ± 5, 4 ± 4 and 5 ± 3 mg/dl respectively (Fig. 2). Although propranolol completely blocked the isoproterenol-induced NEFA elevation ($P < 0.01$), C-4 and C-6 did not ($P > 0.1$). In contrast, there were no significant differences in the blockade of glucose response between propranolol and the two test compounds (Fig. 2B). Their relative potencies in metabolic and cardiac effects were summarized in Table 2.

## DISCUSSION

This study was performed to determine whether

Table 2. Relative potencies of propranolol derivatives in rats

| | Relative potencies | | | |
|---|---|---|---|---|
| | dP/dt | HR | NEFA | Glucose |
| Propranolol | 1.00 | 1.00 | 1.00 | 1.00 |
| C-4 | 1.33 | 4.62 | 0.20 | 0.91 |
| C-6 | 2.50 | 4.62 | 0.60 | 0.89 |

the unusual *in vitro* tissue and receptor specificity of the congener derivatives of propranolol [7] could be reproduced *in vivo*. The *in vitro* testing for the congener derivatives of propranolol included (1) biological assays (blocking isoproterenol-induced cAMP accumulation) and (2) radioligand binding assays. The membrane preparations used in these assays were obtained from S-49 lymphoma cells ($\beta_2$ receptors), rat adipose tissue ($\beta_1$-like receptors), rat heart (>65% $\beta_1$ receptors) and rat lungs (>65% $\beta_2$ receptors). Employing these *in vitro* screens, we observed the most atypical effects with the *p*-toluidide and *p*-trifluoromethyl derivatives of propranolol. The most striking differences were the selectivity for $\beta_1$-adrenergic receptors on heart compared to those on adipose tissue using *in vitro* assays. Therefore, we chose the *p*-toluidide and *p*-trifluoromethyl derivatives of propranolol for the *in vivo* testing.

The pithed rat model was used for the *in vivo* experiments because the preparation is ideal for studying direct cardiac effects of drugs since contribution of the sympathetic tone and autonomic reflex is negligible. The changes in dP/dt and heart rate were therefore predominantly, if not solely, due to isoproterenol $\beta$-adrenergic receptor activation of the heart. The congener derivatives of propranolol blocked the isoproterenol-induced inotropic and chronotropic effects, indicating that the *p*-toluidide and *p*-trifluoromethylanilide derivatives were 4.6-fold more potent than propranolol in reducing heart rate, yet only 1.3- and 2.5-fold more potent than propranolol in reducing the contractile force respectively. Perhaps the differential effects on heart rate over contractility could represent pharmacologic and even clinical advantage over propranolol.

Although the differential effects of these derivatives on heart rate versus contractility have so far been stressed, we were also interested in the *in vitro* separation of their effects on myocardium versus adipose tissue. The determination of lipolysis *in vivo* can be complicated because catecholamine-induced lipolysis in the rat is quite resistant to blockade by classical $\beta$-adrenergic antagonists [13]. Furthermore, atypical or hybrid adrenergic receptors with characteristics of both $\beta_1$- and $\beta_2$-subtypes may mediate lipolysis [14–16], and $\beta$-adrenergic actions may play a role in adipose tissue metabolism [17, 18]. Nevertheless, it has been established that catecholamines produce highly significant increases in plasma NEFA and that the effects of catecholamines on fatty acid mobilization are primarily mediated through $\beta$-adrenergic receptors in adipose tissue [19, 20]. The elevation of plasma NEFA generally is proportional to increased lipolysis at the level of the adipose tissue. A small portion of the increase may originate via activation of lipoprotein lipase [21].

Although propranolol or its derivatives given alone did not affect the basal concentration of plasma NEFA or glucose, propranolol blocked the increase in NEFA caused by isoproterenol. As were predicted by the *in vitro* tests, *p*-toluidide and *p*-trifluoromethylanilide derivatives, however, unlike the progenitors, did not substantially block the lipolysis induced by isoproterenol. Despite their inability to inhibit the isoproterenol effects on adipose tissue, both derivatives completely inhibited the isoproterenol-induced hyperglycemia, as did propranolol. Perhaps these data can be explained on the basis of the compounds having more effect on the typical $\beta$-receptors that mediate gluconeogenesis than on the atypical ones in adipose tissue.

In conclusion, the *in vivo* observations supported the *in vitro* results. These derivatives were more selective for $\beta$-adrenergic receptors on heart than on the $\beta$-adrenoceptors of adipose tissue.

Similarly, it may be possible to derivatize other cell membrane active agonists and antagonists in the same way that we have with $\beta$-adrenergic agonists and antagonists and histamine with the expectation of modifying their potency and, more importantly, their receptor and effect selectivity.

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