Effects of the root of *Polygala tenuifolia* on Catecholamine Biosynthesis in PC12 Cells

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The root of *Polygala tenuifolia* Willdenow (Polygalaceae) is a well known herbal medicine used as an expectorant, tonic or sedative. The constituents of *P. tenuifolia* such as saponins, sapogenins, sugars and xanthones derivatives have been reported (Tang and Eisenbrand, 1992). The PC12 cells derived from rat adrenal pheochromocytoma exhibit many properties of the adrenal medullary chromaffin cells, including the synthesis, storage and secretion of catecholamines (Greene and Rein, 1977; Tischler *et al.*, 1983). The PC12 cells also express tyrosine hydroxylase (TH), the rate-limiting enzyme of the catecholamine biosynthetic pathway (Greene and Rein, 1977). Previously, we have reported that the methanol (MeOH) extracts from several herbal medicines including *P. tenuifolia* have an inhibitory effect on catecholamine biosynthesis in PC12 cells (Lee *et al.*, 1995). In this study, the bioactive butanol (BuOH) fraction was partitioned from the MeOH extract of *P. tenuifolia* and the effects of BuOH fraction on catecholamine content and TH activity in PC12 cells were investigated.

The dried roots of *P. tenuifolia* Willdenow were purchased from Han-Kook Sin Yak Pharm. Co. Ltd. (Taejeon, Korea). A voucher specimen is deposited in the Department of Pharmacology at the College of Pharmacy, Chungbuk National University. All chemicals were of reagent grade. MeOH extract (90 g) which obtained from the dried roots of *P. tenuifolia* was suspended in water (1.5 L) and partitioned three times with dichloromethane (CH_2Cl_2, 1.5 L) and ethylacetate (EtOAc, 1.5 L) successively. The aqueous layer was extracted three times with BuOH (1.5 L). As the solvent was removed under reduced pressure, each extract and the aqueous layer were frozen and dried to a powder (CH_2Cl_2 fraction, 6.3 g; EtOAc fraction, 1.5 g; BuOH fraction, 18.5 g; H_2O fraction, 48.4 g). The PC12 cells were grown routinely as described previously (Tischler *et al.*, 1983). The cells (ca. 1×10^5 cells/cm^2) were treated with each extract (40 μg/ml medium) and then incubated for 48 hrs. The cells (ca. $1.5\text{-}2\times10^5$ cells/cm^2) were harvested and centrifuged. The pellet extract was used for the measurement of catecholamine content and TH activity. Catecholamine content was determined as described previously (Mitsui *et al.*, 1985; Lee *et al.*. 1994). The TH activity was measured by a modification of the method of Nagatsu *et al.* (1979). The conditions of the fluorescence derivatization and HPLC analysis were the same as described previously (Lee *et al.*, 1994). Protein amounts were determined by the method of Lowry *et al.* (1951).

We have previously reported that the MeOH extract of *P. tenuifolia* (40 μg/ml medium) shows an inhibitory effect on dopamine biosynthesis in intracellular PC12 cells (Lee *et al.*, 1994). Therefore, the MeOH extract was successively partitioned into CH_2Cl_2, EtOAc, BuOH and H_2O fractions (Chart 1). Among them, the BuOH fraction (40 μg/ml medium) showed 67.5 % and 52.9 % inhibition on dopamine and norepinephrine content in PC12 cells (Table I). The secretion of catecholamines (dopamine and norepinephrine) into the medium slightly increased upon addition of the BuOH fraction (40 μg/ml medium), but these were not significant (data not shown). The cell viability was examined by trypan blue exclusion test. Solvent fractions used did not show cytotoxicity towards PC12 cells. Therefore, the catecholamine con-

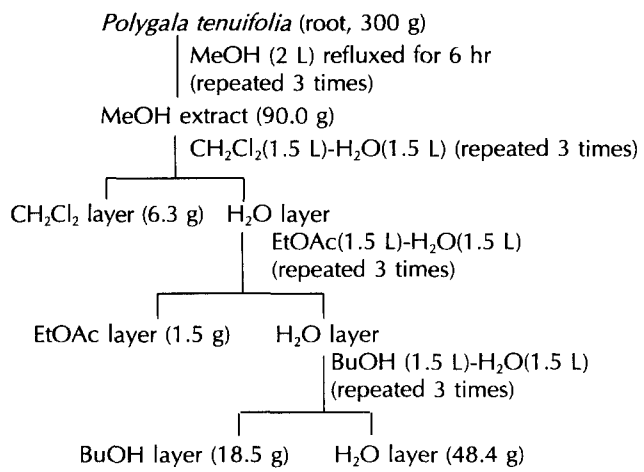

Chart I. Isolation of the active fractions from the roots of *Polygala tenuifolia*.

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Table I. Effects of the root of *P. tenuifolia* on the content of intracellular catecholamines in PC12 cells

Hebal medicine (40 μg/ml medium)	Catecholamine content (% of control)		TH activity (% of control) (nmol/min/mg protein)
	Norepinephrine (pmol/mg protein)	Dopamine (nmol/mg protein)	
Control	87.1±5.1(100)	4.42±0.51(100)	96.4±6.4(100)
CH_2Cl_2 Fr.	87.9±9.8(101)	4.73±1.12(107)	
EtOAc Fr.	65.1±6.4(74.7)*	3.64±1.22(82.4)	
BuOH Fr.	41.0±8.1(47.1)**	1.44±0.30(32.6)**	55.7±7.5(57.8)**
H2O Fr.	76.8±9.8(88.2)	4.25±0.33(96.1)	

Cells were incubated for 24 hrs and replaced by fresh media. The cells were treated with various fractions of *P. tenuifolia* (40 μg/ml medium) and then incubated for 48 hrs. The cells were harvested with PBS, and the catecholamine content and TH activity were measured by HPLC. Results represent the mean±SE of six dishes. Significantly different from the control value: *, $p<0.05$; **, $p<0.01$ (Student's t test).

tent, stored in the cells and secreted into the medium, was significantly reduced by the BuOH fraction in PC 12 cells. TH activity in PC12 cells is responsible for various factors such as c-AMP, dexamethasone, growth factors, protein kinase A and protein kinase C (Lewis *et al.*, 1987; Stachowiak *et al.*, 1988). TH activity was markedly reduced by treatment of the BuOH fraction (42.2% inhibition at 40 μg/ml in the medium; Table I). This result suggests that the BuOH fraction has an inhibitory effect on catecholamine biosynthesis by reducing TH activity in PC12 cells.

Seven intact saponins from the BuOH fraction were isolated and designated as onjisaponins A-G (Sakuma and Shoji, 1981; Sakuma and Shoji, 1982). They are all tenuifolin-28-esters of different oligosaccharides. Thus, we hypothesize that onjisaponin derivatives from the BuOH fraction have an inhibitory effect on catecholamine biosynthesis in PC12 cells. Saponins showed an inhibitory activity against c-AMP phosphodiesterase (Nikaido *et al.*, 1982), suggesting that saponins increase the c-AMP content and activate TH activity. In our experiments, TH activity was reduced by the BuOH fraction in PC12 cells. It is thought that the reason for those differences might be due to the experimental method between enzyme activity *in vitro* and intracellular regulation. The separation of bioactive component(s) from *P. tenuifolia* as well as mechanisms in intracellular PC12 cells need further investigation.

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