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Inhibitory effects of newly synthesized sulfonamide derivatives on calmodulin-dependent phosphodiesterase activity and their modulation of the external ATP-dependent permeability change in mammalian cultured cells

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External ATP causes a passive permeability change in several types of transformed cells and this change is further enhanced by calmodulin antagonists, such as trifluoperazine. However, such drugs also have nonspecific effects on membrane permeability. We have synthesized several new sulfonamide derivatives, which were found to inhibit calmodulin-dependent phosphodiesterase. The drugs also enhanced the ATP-dependent permeability change in CHO-K1 cells, but their effective concentration ranges were wider than those of previously known antagonists, and thus they would be useful for pharmacological use.

The permeability of the plasma membrane in mammalian cells plays an important role in the control of cellular homeostasis as well as of the actions of chemotherapeutic agents. It has been demonstrated that brief exposure of several types of transformed cells in culture, such as B16 melanoma, Hela and CHO-K1 cells, to external ATP markedly increases passive permeability, allowing passage through the plasma membrane of phosphorylated metabolites and ions [1–6]. Untransformed cells, including mouse 3T3 cells and mouse embryo fibroblasts, did not respond to ATP under the same experimental conditions [1–6].

Although the molecular mechanism by which external ATP controls the permeability change is little understood, application of this phenomenon for the in vivo modulation of the membrane permeability for chemotherapeutic drugs like anti-cancer agents would be worthwhile examining. Recently, we and others demonstrated that calmodulin antagonists, such as TFP and W7, enhance the ATP-dependent permeability change in transformed cells [7–10]. These effects on passive permeability were only observed in limited concentration ranges, and a higher concentration of the drug alone increased the membrane permeability, resulting in cell death [8,9]. Therefore, the development of new calmodulin antagonists with wider action spectra would be very useful for modulation of the ATP effect as well as for studying the mechanism.

We report here that newly synthesized sulfonamide derivatives inhibit CaM-dependent PDE activity and also enhance the external ATP-induced permeability change in wider concentration ranges than those of other known antagonists.

Five sulfonamide derivatives (K-6803, K-6807, K-6917, K-6920, K-7027), whose structures are shown in Table I, were synthesized as follows: K-6803 and K-6807 were synthesized from benzenesulfonyl chloride with tetrahydro-*N*-(3-aminopropyl)isoquinoline or 1-(3-aminopropyl)-2,5-dimethyl-4-diphenylmethylpiperazine, respectively. K-6917 was synthesized from 1-benzenesulfonyl-4-(3-isobutoxy-2-hydroxypropyl)piperazine, and was further methylated to produce K6920. K7027 was synthesized from 1-[3-isobutoxy-2-(*N*-benzyl-*N*-methyl)aminopropyl]piperazine with 5-chloronaphthalenesulfonyl chloride. 2-Deoxy[1-^{3}H]-glucose and [2,8-

Abbreviations: TFP, trifluoperazine; W7, *N*-(6-aminohexyl)-5-chloronaphthalenesulfonamide; CaM, calmodulin; PDE, phosphodiesterase.

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^{3}H]cAMP were from. Amersham International. All other chemicals were obtained as in Refs. 7 and 8.

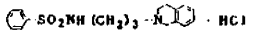
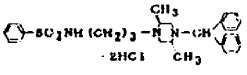
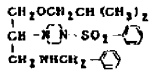
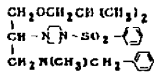
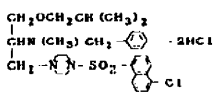
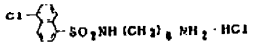
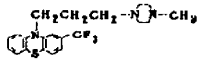
The effects of the examined drugs on PDE (from bovine brain, Sigma) activity were determined by the method of Wallace et al. [11] in the presence of either 50 μM Ca^{2+} or 1 mM EGTA in a reaction mixture containing 10 units/ml calmodulin (from bovine brain. Sigma), 40 mM Tris-HCl (pH 8.0), 3 mM $MgCl_2$ and 2 μM [^{3}H]cAMP (0.3 μCi/ml). Chinese hamster ovary cells. CHO-K1. which had been cultured in Ham's F12 medium supplemented with 10% fetal calf serum as described [4] were labeled for 3 h at 37°C with deoxy[^{3}H]glucose (0.25 μCi/ml, 1 μM) in glucose-free F12 medium containing 10% dialyzed calf serum. The labeled cells were washed twice with 0.15 M NaCl and then incubated at 37°C for 10–15 min with buffer A (0.1 M Tris-HCl (pH 8.2), 0.05 M NaCl, 0.05 mM $CaCl_2$) containing the indicated additions. After incubation, the radioactivity released into the medium was determined with a liquid scintillation counter.

As shown in Table I, four newly synthesized sulfonamide derivatives, K-6807, K-6917, K-6920 and K-7027, inhibited CaM-dependent PDE activity in a dose-dependent manner. The IC_{50} values of these compounds were 25–45 μM in the presence of Ca^{2+}-CaM, while without Ca^{2+} they were more than 100 μM, indicating that the inhibition is Ca^{2+}-dependent. A similar inhibitory effect on CaM-PDE activity was observed with control compounds such as TFP and W7. TFP showed relatively higher CaM-independent inhibition at 100 μM than other sulfonamide derivatives, including W7. One derivative (K-6803), however, showed little inhibitory effect on PDE activity, although its structure is very close to that of K-6807.

TABLE I

Inhibition of CaM-dependent PDE activity by new sulfonamide derivatives, W7 and TFP

Effects of several types of drugs at the indicated concentrations on CaM-PDE activity in the presence or absence of 50 μM Ca^{2+} were determined as described in Methods. The values represent the means of two different experiments and inhibition (%) in the absence of Ca^{2+} is indicated in a parenthesis. IC_{50} means a concentration of the drug required for 50% inhibition of the enzyme activity and IC_{50} values in the absence of Ca^{2+} are also in parentheses.

Compound No.		Inhibition (%)			IC_{50} (μM)
		1 μM	10 μM	100 μM	
K-6803		0 (0)	0 (0)	9 (9)	>100 (>100)
K-6807		13 (9)	47 (19)	52 (23)	43 (>100)
K-6917		4 (4)	36 (0)	71 (15)	25 (>100)
K-6920		0 (0)	12 (5)	70 (23)	46 (>100)
K-7027		0 (1)	45 (13)	59 (24)	23 (>100)
W7		0 (0)	25 (0)	85 (37)	24 (>100)
TFP		0 (2)	56 (14)	86 (54)	8 (81)

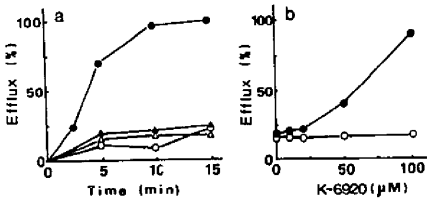


Fig. 1. Induction of a permeability change in CHO-K1 cells by ATP and K-6920. (a) [^{3}H]Deoxyglucose-labeled CHO-K1 cells were incubated at 37°C in buffer A containing the following additions: none, ○; 0.5 mM ATP, ▲; 100 μM K6920, △; 0.5 mM ATP and 100 μM K-6920. ●. After incubation, radioactivity released into the medium was counted and efflux (%) was calculated on the basis of the total radioactivity within the cells which could be extracted with 5% cold TCA ($2.5 \cdot 10^4$ cpm/dish). (b) The labeled CHO-K1 cells were also treated with various concentrations of K-6920 and 0.5 mM ATP at 37°C for 15 min and the efflux (%) was measured.

When CHO-K1 cells labeled with deoxy[^{3}H]glucose are treated with 0.1–0.5 mM ATP and 10–30 μM TFP in buffer A (pH 8.2), a great increase in the efflux of radioactive materials is rapidly induced (Ref. 8 and Table II). A similar permeability change in CHO-K1 cells was induced by the addition of external ATP and an new sulfonamide derivative, K-6920, in a dose-dependent manner (Fig. 1). This permeability change was completed within 10 min incubation and ATP or the drug alone did not induce a permeability change. An ATP analogue, Ado*PP*[NH]*P*, and ADP were also found to have moderate effects on passive permeability in the presence of this compound, but AMP and other nucleotide triphosphates were all ineffective (data not shown). These characteristics of the permeability change with K-6920 in CHO-K1 cells are quite similar to those previously observed with TFP [8].

The ATP-dependent permeability change was also induced by other derivatives, K-6807 and K-6917, which inhibited CaM-PDE activity (Tables I and II). Among them, K-6807 and K-6920 are particularly interesting, since these drugs alone did not affect the passive permeability of CHO-K1 cells at up to 100 μM, whereas K-6917, like TFP and W7, induced a permeability change at more than 50 μM in the absence of ATP. The concentrations of these compounds required for enhancement of the permeability change were well correlated with those for inhibition of PDE activity. Furthermore, K-6803, which had no inhibitory effect on CaM-PDE, was also found to have little effect on the ATP-dependent permeability change. These results together with the previous ones [8–10] suggest the involvement of calmodulin in the control of the ATP-dependent permeability change. K-7027, which has an inhibitory effect on CaM-PDE, had no stimulating effect on the membrane permeability, probably due to the lower penetration of it into the plasma membrane.

TABLE II

Passive permeability change induced by external ATP and various sulfonamide derivatives in CHO-K1 cells

CHO-K1 cells labeled with [^{3}H]deoxyglucose were incubated in buffer A containing various concentrations of the indicated drug in the absence or presence of 0.5 mM ATP at 37°C for 15 min and efflux (%) was measured as described in Fig. 1.

Drugs (μM)		Efflux (%)			
		0	20	50	100
K-6803	−ATP	15.0	n.d.	18.5	18.1
	+ATP	16.5	n.d.	23.2	35.6
K-6807	−ATP	13.8	20.2	19.7	22.1
	+ATP	18.5	58.0	61.2	77.6
K-6917	−ATP	15.0	17.0	38.5	81.0
	+ATP	16.6	78.0	99.5	100
K-6920	−ATP	15.0	15.1	17.2	16.8
	+ATP	16.6	21.0	40.8	90.0
K-7027	−ATP	13.5	13.7	n.d.	16.0
	+ATP	13.3	17.5	n.d.	17.5
TFP	−ATP	n.d.	10.0	68.5	100
	+ATP	n.d.	55.8	98.0	100
W7	−ATP	16.5	18.4	78.5	100
	+ATP	15.7	48.6	100	100

n.d., not determined.

An ATP-dependent permeability change in CHO-K1 cells can be induced when the cellular ATP level is decreased with mitochondrial inhibitors [4], and this membrane change due to ATP and the inhibitor is suppressed when the cellular ATP level is restored by the addition of excess glucose to the medium. The ATP-dependent permeability changes in CHO-K1 cells induced by K-6807, K-6917 and K-6920, like that with TFP [8], were observable even in the presence of excess glucose in the medium, indicating that the effects of these compounds on the permeability change are independent of the cellular ATP concentration (data not shown).

We synthesized new sulfonamide derivatives, which inhibited PDE activity in a Ca^{2+}/CaM-dependent manner. Calmodulin antagonists, such as TFP and chlorpromazine, are known to exhibit high binding affinity to CaM [12], but nonspecific perturbation of the membranes by these drugs due to their hydrophobic properties remains possible, especially at high concentrations [13,14]. In the present study, it was also observed that CaM-independent inhibition of PDE by TFP was apparent at 100 μM, at which concentration TFP also induce a passive permeability change in CHO cells, as determined from the efflux of [^{3}H]deoxyglucose-labeled pools (Refs. 8 and 9; Tables I and II). Such an ATP-independent permeability change was also induced by W7 or K-6917 at more than 50 μM. It is not clarified yet

whether the ATP-independent permeability changes are caused by nonspecific perturbation of the membranes by these drugs. However, two of the newly synthesized sulfonamide derivatives, K-6807 and K-6820, did not induce permeability change at upto 100 μM in the absence of added ATP. In contrast, these drugs together with the ATP greatly induced a permeability change (Fig. 1 and Table II). Although further experiments are required to determine whether these compounds act as calmodulin antagonists, they will be very useful for modulation of the ATP responses of mammalian cells and for studies on the mechanism. Furthermore, several types of transformed cells are selectively injured on treatment with external ATP [7,15,16]. It should be also noted that external ATP has some pronounced effects on various types of normal cells, including mast cells, lymphocytes and macrophages [17–20]. Thus, ATP-treatment would be useful for cancer chemotherapy management but in some limited way. Such studies both in vitro and in vivo are now investigated.

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