EFFECT OF 2-NICOTINAMIDETHYL NITRATE (SG-75) ON MEMBRANE POTENTIALS OF CANINE PURKINJE FIBERS

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Abstract—In canine Purkinje fibers firing spontaneously at a cycle length of about 2.7 sec, 2-nicotinamidethyl nitrate (SG-75) (10^{-6}–10^{-4} M) increased the cycle length and in high concentrations (10^{-5}–10^{-4} M) abolished the spontaneous firing. The increase in cycle length was due entirely to that decrease in slope of the diastolic depolarization which was more marked during late diastole than during early diastole. Neither the maximum diastolic potential nor the take-off potential was changed. When the membrane potential failed to reach the take-off potential, it polarized again after the early diastolic depolarization, in some fibers. The depth of the notch was increased and the peak value of the plateau of the action potential was decreased by SG-75, however, the maximum rate of rise of the action potential remained unchanged. Shortening of the action potential duration was evident when Purkinje fibers were stimulated at a cycle length of 2 sec. These fibers remained excitable at 10^{-4} M SG-75 and when stimulated at a cycle length of 2 sec, the action potential occurred with a duration of less than half of control. These results can be interpreted as being due to an increase in the background K current induced by SG-75.

2-Nicotinamidethyl nitrate (SG-75) is a new coronary vasodilator comparable in vasodilator potency with papaverine (1). Our previous study (2) on the whole dog and isolated blood-perfused heart preparations of the dog revealed that in doses which increased sufficiently coronary blood flow, SG-75 had virtually no effects on sinus rate, atrioventricular (AV) conduction, force of contraction of the ventricular myocardium and myocardial oxygen consumption. However, in large doses, SG-75 depressed force of contraction of the ventricular myocardium, in a manner seen with Ca-antagonistic vasodilators (2). Nevertheless, unlike Ca-antagonistic vasodilators, SG-75 even in large doses impaired little AV conduction (2). Ca-antagonistic vasodilators do impair AV conduction (3–5). Furthermore, SG-75 in large doses shortened the effective refractory period of the ventricular myocardium (6) and in extremely large doses produced ventricular fibrillation in the isolated blood-perfused papillary muscle preparation of the dog (2, 6). SG-75 suppressed ventricular automaticity assumedly derived from that of Purkinje fibers in the dog heart (6). These three actions of SG-75 are uncharacteristic of Ca-antagonistic vasodilators (6–8). Based on such observations we (6) hypothesized that the mechanism of cardiac actions of SG-75 would be an increase in K conductance in cardiac cell membrane. A subsequent electrophysiological study (9) on canine atrial muscle...
fibers showed that such is indeed the case, with application of SG-75 the resting membrane potential approached to the K equilibrium potential and markedly shortened the action potential duration. The present experiments were designed to investigate the effect of SG-75 on membrane potentials of canine Purkinje fibers, since it remains unsettled whether these potentials of canine Purkinje fibers would be altered by SG-75 in such a way as to be expected from the proposed mechanism of action.

**MATERIALS AND METHODS**

Experiments were carried out on false tendons obtained from the right ventricle of the dog heart. Hearts were removed from mongrel dogs weighing about 10 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.), given sodium heparin (100 units/kg, i.v.) and exsanguinated. The false tendon, about 0.3–1 mm in width and about 5 mm in length, was mounted in a 3.5 ml tissue bath and superfused with Tyrode solution at a flow rate of 7 ml/min by virtue of a roller pump (Holter, Model 911). Tyrode solution was of the following composition (mM): NaCl 137; KCl 2.7; CaCl2 1.8; NaH2PO4 0.4; NaHCO3 11.9; glucose 5.5. The solution was equilibrated with 95% O2 and 5% CO2 and its pH was maintained at 7.4. Temperature of the bath was maintained at 37°C.

Glass microcapillaries filled with 3 M KCl and having resistances of 15–20 MΩ were used. After suitable amplification by a DC amplifier with an electrometer tube in input stage (WP Instruments, M707), membrane potentials were displayed on the screen of a cathode-ray oscilloscope (Nihon Kohden, VC7A) and photographed on film. Photographic recordings were projected and on the projected images were measured the following membrane potential variables: cycle length, maximum diastolic potential, amplitude of the upstroke of the action potential, depth of the notch, peak value of the plateau of the action potential, take-off potential, durations of the action potential at 50% repolarization, at 100% repolarization, slope of the early diastolic depolarization, slope of the late diastolic depolarization. The recording of membrane potentials was obtained in the presence of 3×10⁻⁶ M SG-75.

**Fig. 1.** Variables measured on membrane potentials of spontaneously firing canine Purkinje fibers. 1: cycle length; 2: maximum diastolic potential; 3: action potential amplitude; 4: depth of the notch; 5: peak value of the plateau of the action potential; 6: take-off potential; 7: duration of the action potential at 50% repolarization; 8: duration of the action potential at 100% repolarization; 9: slope of the early diastolic depolarization; 10: slope of the late diastolic depolarization. The recording of membrane potentials was obtained in the presence of 3×10⁻⁶ M SG-75.

2-Nicotinamidethyl nitrate (SG-75) (Chugai Pharmaceutical Co.) was used. The drug was dissolved in 0.9% saline at desired concentrations. After a 1 hr equilibration of the preparation with Tyrode solution, control recordings of membrane...
potentials were obtained in drug-free Tyrode solution and recordings under drug action were obtained 10 min after exposure of the preparation to SG-75.

Values are given in terms of mean±S.E. Difference between mean values was analyzed with Student’s t-test. Difference was judged to be significant when p values were less than 0.05.

RESULTS

Effects on membrane potentials of spontaneously firing Purkinje fibers: Experiments were carried out on 11 false tendons. In Fig. 2 are shown typical recordings of membrane potentials obtained by continuous impalement of one and the same Purkinje fiber before (control) and during exposure of a false tendon to increasing concentrations of SG-75 from $10^{-6}$ to $10^{-4}$ M. In Table 1 are summarized membrane potential variables obtained from the 11 false tendons. When not stimulated, the 11 false tendons fired spontaneously at the mean cycle length of 2.7±0.5 sec (at the mean rate of about 0.37 Hz). With exposure of the false tendons to SG-75 from $10^{-6}$ to $10^{-4}$ M, the cycle length of spontaneous firing of Purkinje fibers increased in a concentration-dependent manner up to 6.1±1.8 sec at $10^{-5}$ M (Table 1). Two of the 11 false tendons ceased spontaneous firing at $10^{-5}$ M and the remaining 9 at $10^{-4}$ M SG-75.

The increase in cycle length of spontaneous firing of Purkinje fibers produced by $10^{-6}$--$10^{-5}$ M SG-75 was due entirely to an increase in diastolic interval, which was in turn due to a decrease in slope of diastolic depolarization (Fig. 2). The decrease in slope of the diastolic depolarization, however, was greater during late diastole than during early diastole (up to about 1.5 sec) (Fig. 2 and Table 1).

The early diastolic depolarization, which started from the maximum diastolic potential of $-92--94$ mV with a relatively steep slope and gave a way to the late diastolic depolarization at about $-80$ mV, was not so markedly depressed by SG-75 as the late diastolic depolarization (Table 1). In contrast, the late diastolic depolarization, which started at $-80$ mV with a flatter slope following the early one and gave a way to the take-off potential of $-71--75$ mV with increasing the slope, was much more depressed by SG-75 than the early diastolic depolarization. In other words, in the presence of SG-75 it appeared to be difficult for the membrane potential to depolarize further to reach the take-off potential of $-71--75$ mV, although the membrane potential depolarized rather rapidly during early diastole. When the membrane potential failed to reach the take-off potential, it polarized again down to about $-80$ mV and the diastolic depolarization was renewed. Such phenomena were observed with $10^{-6}$--$10^{-5}$ M SG-75 as shown in Fig. 3B. With $10^{-5}$ M SG-75, repolarization of the membrane potential during the course of the diastolic depolarization was not so marked as that shown in Fig. 3B but the membrane potential undulated 2–3 times around $-80$ mV following the early diastolic depolarization before it eventually reached the take-off potential as shown in Fig. 3C.

![Fig. 2. Effect of SG-75 on membrane potentials of a spontaneously firing canine Purkinje fiber. A: before exposure to SG-75 (control); B: 10-min exposure to $10^{-6}$ M SG-75; C: 10-min exposure to $10^{-5}$ M SG-75; D: 10-min exposure to $10^{-4}$ M SG-75.](image-url)
Table 1. Effect of SG-75 on membrane potential variables of spontaneously firing Purkinje fibers

| C (M) | N  | CL (sec) | MDP (mV) | SEDD (mV/sec) | SLDD (mV/sec) | TOP (mV) | APA (mV) | Vmax (V/sec) | Notch (mV) | Plateau (msec) | APD50 (msec) | APD100 (msec) |
|-------|----|----------|----------|---------------|---------------|----------|----------|--------------|------------|----------------|--------------|--------------|
|       |    |          |          |               |               |          |          |              |            |                |              |              |
| Control | 11 | 2.7 ± 0.5 | -92.3 ± 2.5 | 12.2 ± 1.1 | 2.7a ± 0.9 | -71.5 ± 4.9 | 11.6 ± 4.6 | 342 ± 72 | -0.2 ± 2.7 | +3.2 ± 2.5 | ±20 ± 26 | ±20 ± 26 |
| 10^-6  | 11 | 4.3 ± 1 | -94.6 ± 1.4 | 10.6 ± 1.1 | 1.3d ± 0.4 | -70.9 ± 3.9 | 114.6 ± 5.2 | 319b ± 175 | -3.6ef ± 3.0 | -0.5 ± 2.8 | ±23 ± 50 | ±23 ± 50 |
| 10^-5  | 9  | 6.1 ± 1.8 | -94.1 ± 2.0 | 10.2d ± 1.2 | 0.9c,d ± 0.3 | -73.7 ± 4.2 | 115.8 ± 6.0 | 315b ± 66 | -7.0c ± 3.2 | -3.6f ± 2.5 | ±31 ± 36 | ±31 ± 36 |
| 10^-4  | 7  | Arrest   | -80.5b ± 2.6 |               |               |          |          |              |            |                |              |              |

C: concentration of SG-75; N: number of fibers; CL: cycle length; MDP: maximum diastolic potential; SEDD: slope of the early diastolic depolarization; SLDD: slope of the late diastolic depolarization; TOP: take-off potential; APA: action potential amplitude; Vmax: maximum rate of rise of the action potential; Notch: depth of notch of the action potential; Plateau: peak value of the plateau of the action potential; APD50: duration of the action potential at 50% repolarization; APD100: duration of the action potential at 100% repolarization. Values are mean±S.E. a N=6; b N=7; c N=5; d p<0.05; e p<0.01; f p<0.001 compared with corresponding control values.
With $10^{-4}$ M SG-75, the membrane potential failed to reach the take-off potential after its undulatory changes and became stable at the level of about $-80$ mV (Fig. 2D and Table 1).

Fig. 3. Effect of SG-75 on membrane potentials of another spontaneously firing canine Purkinje fiber. A: before exposure to SG-75 (control); B: 10-min exposure to $10^{-6}$ M SG-75; C: 10-min exposure to $10^{-5}$ M SG-75.

Fig. 4. Effect of SG-75 on membrane potentials of a canine Purkinje fiber stimulated at a cycle length of 2 sec. A: before exposure to SG-75; B: 10-min exposure to $10^{-6}$ M SG-75; C: 10-min exposure to $10^{-5}$ M SG-75.

With $10^{-4}$ M SG-75, the membrane potential failed to reach the take-off potential after its undulatory changes and became stable at the level of about $-80$ mV (Fig. 2D and Table 1).

Other striking changes in membrane potential variables produced by SG-75 were an increase in depth of the notch between the early repolarization and the plateau of the action potential and a decrease in peak value of the plateau. The mean depth of the notch measured was $-0.2 \pm 2.7$ mV ($n=11$) before exposure to SG-75 (control), and $-3.6 \pm 3.0$ mV ($n=11$) at $10^{-6}$ M SG-75 and $-7.0 \pm 3.2$ mV ($n=9$) at $10^{-5}$ M SG-75, the latter two values being significantly different from the pre-drug value ($p<0.01$) (Table 1). The mean peak value of the plateau was $+3.2 \pm 2.5$ mV ($n=11$) in control, and $-0.5 \pm 2.8$ mV ($n=11$) at $10^{-6}$ M SG-75 and $-3.6 \pm 2.5$ mV ($n=9$) at $10^{-5}$ M SG-75, the latter two values being significantly different from the pre-drug value ($p<0.01$).

The durations of the action potential at 50% and 100% repolarization were reduced by $10^{-5}$ M SG-75 to about 72% and 92% of the pre-drug values but the latter change was not significant (Table 1). The maximum diastolic potential, and the take-off potential, the amplitude and the maximum rate of rise of the action potential of spontaneously firing Purkinje fibers were not significantly altered by SG-75 in a concentration range of $10^{-6}$--$10^{-5}$ M (Table 1).

Effects of SG-75 on membrane potentials of Purkinje fibers stimulated at a cycle length of 2 sec: When false tendons were stimulated at a fixed cycle length of 2 sec (0.5 Hz), Purkinje fibers responded to stimulation even in the presence of SG-75 in a concentration as high as $10^{-4}$ M in which...
Purkinje fibers ceased to fire spontaneously. Figure 4 shows typical recordings obtained from one and the same Purkinje fiber with increasing concentrations of SG-75 up to $10^{-4}$ M, and summarized results are presented in Table 2.

One of marked changes in membrane potential variables produced by SG-75 was a dose-dependent decrease in slope of the diastolic depolarization (Fig. 4 and Table 2) as observed in spontaneously firing Purkinje fibers. However, it should be noted that in Purkinje fibers stimulated at a cycle length of 2 sec, the diastolic depolarization corresponded to only the early phase of the diastolic depolarization of spontaneously firing Purkinje fibers. With $10^{-4}$ M SG-75 the slope of the diastolic depolarization became so flat that it appeared to be abolished (Fig. 4).

Shortening by SG-75 of the action potential duration was also one of marked changes in membrane potential variables produced by SG-75. In spontaneously firing Purkinje fibers the action potential duration was shortened by $10^{-3}$ M SG-75 significantly only at 50% repolarization but not at 100% repolarization, at which time the cycle length increased from about 2.7 sec in control to about 6.1 sec. However, in Purkinje fibers stimulated at a cycle length of 2 sec, the same concentration of SG-75 produced significant shortening of the action potential duration at both 50% and 100% repolarization (Table 2). Since Purkinje fibers still remained normally excitable with $10^{-4}$ M SG-75, a concentration of which Purkinje fibers ceased to fire spontaneously, the pronounced effect of this drug in shortening of the action potential duration was observed; the action potential duration became less than half of the pre-drug value (Fig. 4 and Table 2).

The peak value of the plateau of the action potential was also decreased by SG-75 (Fig. 4), as in spontaneously firing fibers. The maximum diastolic potential, and the amplitude and the maximum rate of rise of the action potential were not altered significantly by SG-75 in concentrations up to $10^{-4}$ M (Fig. 4 and Table 2). In some fibers, however, the maximum diastolic potential was slightly reduced by SG-75 in the highest concentration ($10^{-4}$ M) (Fig. 4C).

### DISCUSSION

In the present experiments, SG-75 in concentrations of $10^{-6}$–$10^{-3}$ M increased the cycle length of spontaneous firing of canine Purkinje fibers. This observation is consistent with the finding obtained in the previous experiments (6) that SG-75 reduced the rate of ventricular automaticity in the isolated blood-perfused papillary muscle preparation of the dog. The increase in cycle length of

### Table 2. Effect of SG-75 on membrane potential variables of canine Purkinje fibers stimulated at a cycle length of 2 sec

| C (M) | N | MDP (mV) | SDD (mV/sec) | APA (mV) | $\dot{V}_{\text{max}}$ (V/sec) | APD50 (msec) | APD100 (msec) |
|-------|---|----------|--------------|---------|----------------|-------------|-------------|
| Control | 7 | $101 \pm 2$ | $5.3 \pm 0.9$ | $130 \pm 3$ | $552 \pm 70$ | $355 \pm 19$ | $552 \pm 26$ |
| $10^{-6}$ | 7 | $101 \pm 2$ | $4.8 \pm 0.7$ | $134 \pm 3$ | $560 \pm 66$ | $343 \pm 18^a$ | $532 \pm 28$ |
| $10^{-5}$ | 7 | $104 \pm 2$ | $4.3 \pm 0.8^b$ | $134 \pm 3$ | $571 \pm 69$ | $312 \pm 18^b$ | $491 \pm 21^b$ |
| $10^{-4}$ | 7 | $98 \pm 2$ | $2.7 \pm 0.7^b$ | $128 \pm 3$ | $556 \pm 66$ | $118 \pm 24^c$ | $235 \pm 22^c$ |

C: concentration of SG-75; N: number of fibers; MDP: maximum diastolic potential; APA: action potential amplitude; $\dot{V}_{\text{max}}$: maximum rate of rise of the action potential; APD50: duration of the action potential at 50% repolarization; APD100: duration of the action potential at 100% repolarization. Values are mean±S.E. $^a$ p < 0.05; $^b$ p < 0.01; $^c$ p < 0.001 compared with corresponding control values.
spontaneous firing was entirely due to the prolongation of diastolic intervals which was primarily produced by reduction of the slope of the diastolic depolarization particularly during late diastole. Other determinants of cycle length of spontaneous firing, i.e., the take-off potential, the maximum diastolic potential and the duration of the action potential at 100% repolarization remained unchanged by SG-75 in concentrations up to 10^{-5} M. Reduction of the slope of the diastolic depolarization was also produced by SG-75 in Purkinje fibers stimulated at a cycle length of 2 sec.

Another effect characteristic of 10^{-5} and 10^{-4} M SG-75 was shortening of the duration of the action potential at 50% and 100% repolarization as observed in Purkinje fibers stimulated at a cycle length of 2 sec. The results are consistent with those observed in canine left atrial muscle fibers in a previous study (9). We have ascribed shortening of the duration of the action potential of atrial muscle fibers produced by SG-75 to an increase in membrane K conductance in these fibers (9). Shortening of the duration of the action potential of Purkinje fibers produced by SG-75 can also be attributed to the same mechanism.

In canine atrial muscle fibers, with application of SG-75 in concentrations of 10^{-5}–10^{-4} M the resting membrane potential hyperpolarized and approached to K equilibrium potentials (9). This implies that SG-75 would increase a time-independent background K current, i_{k1} (10) rather than time-dependent K currents. If we try to interpret the effects of SG-75 on membrane potential variables by a single mechanism of action, the shortening of the duration of the action potential of Purkinje fibers produced by SG-75 can also be ascribed to the increase in i_{k1} rather than time-dependent K currents. The K current i_{k1} plays a large role in repolarization (11). In this respect it was also of interest that SG-75 affected differentially the diastolic depolarization between early diastole and late diastole; the slope of the early diastolic depolarization was less inhibited by SG-75 than that of the late diastolic depolarization. Furthermore, in the present experiments when the membrane potential failed to reach the take-off potential following the diastolic depolarization in the presence of 10^{-5} M SG-75, the potential polarized again from about −75 mV down to about −80 mV (Fig. 3B). It is claimed that the diastolic depolarization of Purkinje fibers is produced initially (up to about 1.5 sec) by a time-dependent decrease in an outward K current, i_{k2}, and afterwards by its voltage-dependent decrease due to the inward-rectifying property of the i_{k2} channel in the presence of inward background currents (12). Resistance of the early diastolic depolarization (up to about 1.3 sec) to the inhibitory action of SG-75 implies that the kinetics of i_{k2} would not be changed by SG-75. Although the late diastolic depolarization is interpreted as being mainly due to the inward-rectifying property of the i_{k2} channel beyond −75 mV (12), contribution of a voltage-dependent decrease in i_{k4} to the late diastolic depolarization cannot be overlooked, since the i_{k4} channel has also the inward-rectifying property beyond −80 mV (13). Moreover, in Purkinje fibers, at any membrane potential, i_{k4} is usually twice as large as the maximal value of i_{k2} (12). Thus, if SG-75 does increase i_{k4}, its rather preferential inhibition of the late diastolic depolarization can be interpreted. Virtual abolition of the diastolic depolarization as observed with 10^{-4} M SG-75 in Purkinje fibers stimulated at a cycle length of 2 sec would also be due to an increase in i_{k4}.

In the present experiments, the peak value of the plateau of the action potential of Purkinje fibers was reduced by SG-75. The slow inward current reportedly plays an
important role in the formation of the plateau (14, 15). Thus, it is possible that SG-75 reduced the peak value of the plateau by inhibiting the slow inward current. The present experiments do not rule out such a possibility. Nevertheless, as suggested in the previous paper (9), the decrease in the slow inward current, if any, would be secondary to the increased K conductance.

In keeping with the reduction of the peak value of the plateau, the depth of the notch was also increased by SG-75. In Purkinje fibers, interruption of the early repolarization by the decrease in $i_{k1}$ due to the inward-rectifying property of the $i_{k1}$ channel at the depolarized level and by the activation of the slow inward current, results in a notch (16). The early repolarization is formed by the rapid inactivation of the fast inward Na current and by the activation of an early outward current, called the positive dynamic current by Peper and Trautwien (17). Thus, the depth of the notch can be increased either by an increase in this positive dynamic current or an increase in $i_{k1}$. Initially Cl ions were thought to be involved in the positive dynamic current (18, 19). However, recently it is suggested that this current may be carried by K ions (20, 21). Whatever ions may be involved in the positive dynamic current, it appears unlikely that SG-75 increased the depth of the notch by affecting membrane Cl conductance. In a previous study, the effect of SG-75 on membrane potential variables in atrial muscle fibers was not altered by replacement of Cl ions by isethionate ions. Alternatively, it appears likely that an increase in $i_{k1}$ produced by SG-75 also increased the depth of the notch.

There is also the question of why the resting membrane potential observed when spontaneous firing of Purkinje fibers ceased with $10^{-4}$ M SG-75 was more positive by about 14 mV than the maximum diastolic potential (−80 mV against −94 mV). This can be interpreted in such a way that in a state in which activation of $i_{k2}$ ceased with abolition of the action potential, contribution of Na and Cl ions to the resting membrane potential would be greater. Standstill of the membrane potential of Purkinje fibers at the level more positive than the maximum diastolic potential has been observed with ACh (22).

The effects of SG-75 in concentrations of $10^{-8}$-10$^{-5}$ M on membrane potentials of Purkinje fibers resemble those of lidocaine in a similar concentration range (23). However, SG-75 differs from lidocaine in the respect that SG-75 has no inhibitory effect on the fast inward Na current. This was evidenced by the findings that even with increasing concentrations up to $10^{-4}$ M SG-75 failed to reduce the maximum rate of rise of the action potential in Purkinje fibers, in the present experiments, and also in atrial muscle fibers in the previous study (9). Lidocaine at a concentration of $10^{-4}$ M has been shown to decrease the maximum rate of rise of the action potential of Purkinje fibers (23).

The effect of SG-75 in reducing the rate of automaticity of Purkinje fibers is not antagonized by atropine (6) and its effect on membrane potential variables in atrial muscle fibers is not altered by atropine. Thus, the effect of SG-75 on cardiac muscle is independent of muscarinic acetylcholine receptors. The effectiveness of SG-75 on atrial and ventricular muscle and Purkinje fibers of the dog heart indicates that SG-75 may be a useful pharmacological tool to increase the background K current in cardiac muscle.

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