POTENTIATION OF THE ALGOCGENIC ACTION OF BRADYKININ BY AN INHIBITOR OF ANGIOTENSIN I CONVERTING ENZYME, CAPTOPRIL (SQ 14,225)

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Abstract—Experiments were conducted on 11 loosely restrained, conscious dogs which responded with vocalization in a dose-dependent manner to bradykinin (0.6–10 nmol) or acetylcholine (0.1–10 μmol) injected into the femoral or the mesenteric artery through a chronically indwelling arterial catheter. Vocalization was taken as being to reflect the algogenic action of these substances. Administration of captopril (1 mg/kg, i.v.) potentiated the algogenic action of bradykinin but not that of acetylcholine injected into either artery. The magnitude of potentiation of the algogenic action was about 3-fold for bradykinin injected into the femoral artery and about 10-fold for that injected into the mesenteric artery. The potentiation can be interpreted in terms of inhibition of kininase II by captopril. The difference in potentiation by captopril of the algogenic action of bradykinin injected into the two arteries may reflect the difference in kininase II activity in the two arterial beds. Possible involvement of E-series prostaglandins in the potentiation was also discussed.

D-3-mercapto-2-methylpropanoyl-L-proline (SQ 14,225 or captopril) is an orally active inhibitor of angiotensin I converting enzyme developed in the hope of its being useful in the diagnosis and treatment of patients with renin-dependent hypertension (1–3). SQ 14,225 is capable of producing a sustained fall of blood pressure in renin-dependent models of hypertension (4, 5). Since angiotensin I converting enzyme also inactivates bradykinin as kininase II (6), SQ 14,225 augments the vasodepressor effect of intravenous bradykinin (1–3). This is true for the vasodilator effects of bradykinin or kallikrein injected intra-arterially (7). Thus, it can be anticipated that SQ 14,225 administered systemically would augment the algogenic effect of bradykinin. The present experiments were performed to elucidate this point. Since different mechanisms have been proposed to operate in the algogenic action of bradykinin in the hind limb (a somatic area) and in the gut (a visceral area) (8) experiments were conducted in the two areas. Vocalization of conscious dogs to intra-arterial injection of algogenic substances is taken to reflect the algogenic action of these substances.

MATERIALS AND METHODS

Experiments were performed on 11 mongrel dogs of either sex, weighing 6–8 kg. Polyvinyl tubing of about 0.6 mm in outer diameter was implanted in the femoral artery or in a branch of the ileal or jejunal (called simply mesenteric bellow) artery under pentobarbitone anaesthesia 4 or 5 days prior to the experiment. During the experiment the animals
were loosely restrained with a hammock. Vocalization was picked up with a non-directional microphone placed about 30 cm in front of the nostril of the animal, and recorded on magnetic tapes. Throughout the experiment vocalization was simultaneously displayed as an integrated tracing through an RC circuit with a time constant of 2 sec. Details of the technique of implantation of the arterial catheter and experimental procedures were described previously (8, 9). The compounds used were acetylcholine (ACh) chloride (Daiichi Seiyaku), bradykinin (Protein Research Foundation) and D-3-mercapto-2-methylpropanoyl-L-proline (SQ 14,225 or captopril) (the Squib Institute of Medical Research). ACh and bradykinin were dissolved in 0.9% saline and diluted with 0.9% saline to the desired concentrations. SQ 14,225 was dissolved in 100 mM phosphate buffer solution prepared from redistilled water and saturated with nitrogen gas. pH of the solution of SQ 14,225 thus made up was about 7. Solutions of algogenic substances were injected into the catheter in a volume of 0.1 ml and flushed into the artery with 0.5 ml of 0.9% warm saline at a rate of 0.1 ml/sec. SQ 14,225 (1 mg/kg) was administered into the cephalic vein of the dog in a single bolus.

Dose-response relations for vocalization were constructed in the following way. After each experiment, tapes were played back and vocalization responses were reproduced as integrated tracings through the circuit described above on the chart in such a way that the largest vocalization response spanned the full range of pen excursion. In records thus obtained, areas circumscribed by a base line and integrated tracings were measured planimetrically. Since 10 μmol of ACh, whether injected into the femoral artery or into the mesenteric artery, gave the largest response in most dogs, the size (viz., area) of the response to a given dose of ACh was expressed as a percentage of the size of the response to 10 μmol of ACh. The size of response to a given dose of bradykinin injected into the femoral artery was expressed as a percentage of the size of the response to 10 nmol of bradykinin into the same artery and the size of the response to a given dose of bradykinin injected into the mesenteric artery was expressed as a percentage of 6 nmol of bradykinin injected into the same artery; 10 and 6 nmol of bradykinin, when injected into respective arteries, gave the largest responses in most dogs. Measurements of latency and duration of vocalization were also made on the integrated tracings.

All values are given in terms of mean ± S.E., unless otherwise stated. Significance in differences between mean values was evaluated by Student's t-test, and expressed as p values. The difference was judged to be significant when the p value was less than 0.05.

RESULTS

Since in dogs 1 mg/kg, i.v. of SQ 14,225 is known to be sufficient to potentiate the vasodilator effect of intra-arterial bradykinin on the vascular bed of the tongue (7), this dose was chosen in the experiments described below.

Potentiation by SQ 14,225 of the algogenic action of bradykinin injected into the femoral artery (in a somatic area): The effect of SQ 14,225 (1 mg/kg, i.v.) on the algogenic action of bradykinin was investigated on 5 dogs which responded with vocalization in a dose-dependent manner to bradykinin (1–10 nmol) injected into the femoral artery (Figs. 1 and 2).
FIG. 1. Upper panel: integrated tracings of vocalization responses of a dog to graded doses of bradykinin injected into the femoral artery. Lower panel: potentiation by SQ 14,225 (1 mg/kg, i.v.) of vocalization responses to the subthreshold test dose (1 nmol) of bradykinin. SQ 14,225 was injected into the cephalic vein of the dog in a single bolus. Time markers in 10 sec. Solid rectangles under the base line indicate a period of flushing of injected materials and numerals, such as 14:35, refer to time of flushing.

FIG. 2. Left panel: dose-response relation for vocalization to bradykinin injected into the femoral artery. Areas of integrated tracings of vocalization responses to a given dose of bradykinin are expressed as percentages of those to 10 nmol of bradykinin. Vertical lines represent S.E. of the mean. N refers to the number of the dogs. Right panel: potentiation by SQ 14,225 (1 mg/kg, i.v.) of vocalization responses to the test doses of bradykinin, 0.9 ± 0.3 (S.D.) nmol. Areas of vocalization responses to the test doses are expressed as percentages of those to 10 nmol of bradykinin in control experiments. N refers to the number of experiments. Further explanation in text.
In control experiments on the 5 dogs, the mean threshold dose of bradykinin for vocalization was $2.1 \pm 0.5 \text{ nmol (n=5)}$. In each dog a subthreshold dose for vocalization was chosen as a test dose and it was confirmed to be subthreshold for vocalization before administration of SQ 14,225 (Fig. 1). The test dose was injected into the femoral artery 3–5 min after a single injection of SQ 14,225 (1 mg/kg, i.v.). As shown in Fig. 1, the subthreshold test dose (1nmol) of bradykinin injected 5 min after SQ 14,225 produced vocalization comparable in size and latency to that produced by a dose between 3 and 6 nmol of bradykinin in the control experiment. Summarized data obtained from similar 5 experiments on the 5 dogs are shown in Fig. 2. The mean test dose of bradykinin was $0.9 \pm 0.3 \text{ (S.D.) nmol}$ and when given $3.5 \pm 1.2 \text{ (S.D.) min}$ after SQ 14,225 (1 mg/kg, i.v.), it was able to produce vocalization comparable in size to that produced by 3 nmol of bradykinin in the control experiments. Furthermore, the latency of vocalization evoked by the test dose of bradykinin after SQ 14,225 was $19.6 \pm 2.7 \text{ sec}$ and that by 3 nmol of bradykinin in the control experiments was $17.6 \pm 2.1 \text{ sec}$, and there was no significant difference between the two values ($p>0.8$). There was also no significant difference between the duration of vocalization evoked by the test dose of bradykinin and that evoked by 3 nmol of bradykinin ($19.6 \pm 2.4 \text{ sec}$ as against $19.6 \pm 3.7 \text{ sec}$ in control, $p>0.8$). Thus, SQ 14,225 shifted the dose-response relation to bradykinin for vocalization to the left by a factor of about 3. In 2 of the 5 dogs the time course of the potentiation was pursued by injecting bradykinin again about 25 and 40 min after administration of SQ 14,225. Although the degree of potentiation gradually declined towards 40 min, the potentiation appeared to last further.

Potentiation by SQ 14,225 (i.v.) of the algogenic action of bradykinin administered into the mesenteric artery (a visceral area): Experiments were carried out on 5 other dogs which vocalized to bradykinin (0.6–10 nmol) injected into the mesenteric artery. The dose-response relation to bradykinin for vocalization obtained from one of such experiments is shown in Fig. 3 and summarized data as such are shown in Fig. 4. The mean threshold dose of bradykinin for vocalization was $1.6 \pm 0.6 \text{ nmol (n=5)}$. The mean subthreshold dose, $0.6 \pm 0.4 \text{ (S.D.) nmol}$, of bradykinin was chosen as the test dose. When given $3.8 \pm 0.8 \text{ (S.D.) min (n=5)}$ after SQ 14,225 (1 mg/kg, i.v.), the test dose of bradykinin evoked vocalization comparable in size to that evoked by 6 nmol of bradykinin in the control experiments (Figs. 3 and 4). The latency of vocalization elicited by the test dose of bradykinin after SQ 14,225 (12.8±3.1 sec (n=5)) was not significantly different from the latency of vocalization evoked by 6 nmol of bradykinin in the control experiments, $15.7 \pm 0.3 \text{ sec (n=5)}$ ($p>0.3$). The mean duration of vocalization in response to the test dose of bradykinin after SQ 14,225 was $26.4 \pm 3.8 \text{ sec}$ and that of 6 nmol of bradykinin $27.8 \pm 3.4 \text{ sec}$, respectively, and there was also no significant difference between the two mean values ($p>0.4$). Thus, the dose-response relation for vocalization to bradykinin injected into the mesenteric artery was shifted by SQ 14,225 to the left by a factor of about 10. The time course of the potentiation was pursued in 3 of the 5 dogs. The degree of potentiation gradually declined towards 35 min after administration of SQ 14,225 (Fig. 3).

Absence of potentiation by SQ 14,225 (i.v.) of the algogenic action of ACh injected into
FIG. 3. Upper panel: vocalization responses of a dog to graded doses of bradykinin injected into the mesenteric artery. Lower panel: potentiation by SQ 14,225 (1 mg/kg, i.v.) of vocalization responses to the test dose (0.3 nmol) of bradykinin. SQ 14,225 was injected into the cephalic vein of the dog in a single bolus. Notations are the same as those in Fig. 1.

FIG. 4. Left panel: dose-response relation for vocalization to bradykinin injected into the mesenteric artery. Areas of integrated tracings of vocalization responses to a given dose of bradykinin are expressed as percentages of those to 6 nmol of bradykinin. N refers to the number of dogs. Right panel: potentiation by SQ 14,225 (1 mg/kg, i.v.) of vocalization responses to test doses of bradykinin, 0.6 ± 0.4 (S.D.) nmol. Areas of vocalization responses to the test doses of bradykinin are expressed as percentages of those to 6 nmol of bradykinin in control experiments. N refers to the number of experiments. Other details are as in Fig. 2.
the femoral artery or into the mesenteric artery: Whether or not the potentiating effect of SQ 14,225 would be extended to the algogenic action of ACh was examined in the same

**Fig. 5.** Upper panel: vocalization responses of a dog to graded doses of ACh injected into the femoral artery. Lower panel: absence of potentiation by SQ 14,225 (1 mg/kg, i.v.) of vocalization responses to the test dose (1 μmol) of ACh. SQ 14,225 was injected into the cephalic vein of the dog in a single bolus. Notations are the same as those in Fig. 1.

**Fig. 6.** Upper panel: vocalization responses of a dog to graded doses of ACh injected into the mesenteric artery. Lower panel: absence of potentiation by SQ 14,225 (1 mg/kg, i.v.) of vocalization responses to the test dose (1 μmol) of ACh. SQ 14,225 was injected into the cephalic vein of the dog in a single bolus. Notations are the same as those in Fig. 1.
4 dogs that were subjected to the experiments with bradykinin injected into the femoral artery and on another dog. The 5 animals responded with vocalization in a dose-dependent manner to ACh (0.1 to 10 μmol) injected into the femoral artery. The threshold dose of ACh was 1.6±0.6 μmol (n=5) and the subthreshold test dose of ACh chosen was 0.6±0.4 (S.D.) μmol. In all of the 5 dogs the test dose of ACh injected into the femoral artery 3–5 min after administration of SQ 14,225 (1 mg/kg, i.v.) failed to elicit vocalization. A typical experiment is shown in Fig. 5. Essentially similar results were obtained on the algogenic action of ACh administered into the mesenteric artery with SQ 14,225 (1 mg/kg, i.v.) Fig. 6. Experiments were carried on 5 dogs, 3 of which were the same dogs that were subjected to the experiments on bradykinin in the femoral artery and one of which was given bradykinin in the mesenteric artery and the remaining one was new. All the 5 dogs vocalized in a dose-dependent manner in response to ACh (1–10 pmol) injected into the mesenteric artery. The threshold dose of ACh was 2.2±0.5 pmol (n=5) and the subthreshold test dose of ACh chosen was 0.8±0.2 (S.D.) pmol. The test dose administered 3–5 min after SQ 14,225 (1 mg/kg, i.v.) failed to evoke vocalization. Thus, the potentiating effect of SQ 14,225 was not extended to the algogenic activity of ACh.

**DISCUSSION**

As expected the present experiments clearly demonstrated that SQ 14,225 potentiated the algogenic action of bradykinin injected into the femoral artery (in the hind limb, a somatic area) as well as that injected into the mesenteric artery (in the gut, a visceral area). The potentiating effect of SQ 14,225 spared the algogenic action of ACh in both the hind limb and the gut. Thus, the mechanism for potentiation by SQ 14,225 of the algogenic action of bradykinin appears to be similar to that for potentiation by the same compound of the vasodepressor (1-3) or the vasodilator effect (7) of bradykinin. The proposed mechanism is that exogenous bradykinin is less inactivated and remains in high concentrations at its site of action because kininase II which inactivates bradykinin is inhibited by SQ 14,225. Kininase II and angiotensin I converting enzyme have been identified to be the same enzyme (6). However, the possibility that a part of the potentiating effect of SQ 14,225 might be due to the enhanced release of E-series prostaglandins cannot be ruled out. It is known that bradykinin releases E-series prostaglandins from tissues (10–12) and that E-series prostaglandins sensitize endings of pain fibres in somatic (12, 13) and visceral areas (11, 13). In the presence of SQ 14,225 E-series prostaglandins are released in accordance with the remaining levels of bradykinin and the endings of pain fibres are sensitized accordingly.

In the present experiments potentiation by intravenous SQ 14,225 (1 mg/kg) of the algogenic action of bradykinin was different between the hind limb and the gut; 3-fold augmentation in the hind limb as opposed to 10-fold augmentation in the gut. To what can this difference be ascribed? In the previous study (8) one (Taira) of us has proposed that different mechanisms would operate in the algogenic action of biogenic algogenic substances like ACh, bradykinin and histamine between the hind limb and the gut. In the hind limb, the algogenic action of biogenic algogenic substances would be ascribed to their
direct excitant action on pain fibres, whereas in the gut, a tubular organ composed of visceral 
smooth muscle, the algogenic action of these substances would be ascribed to their stimulant 
action on smooth muscle, vigorous contractions of which would indirectly excite pain fibres 
(8). Differences in potentiation by SQ 14,225 of the algogenic action of bradykinin may 
be explained on the basis of the dissimilar mode of action of bradykinin in the two areas. However, another explanation appears to be more plausible; the disparity may reflect the 
difference in the activity of kininase II in the two areas. In the mesenteric arterial bed, 
kininase II activity just may be greater than in the femoral arterial bed. However, since 
there are no data comparing the activity of kininase II in the two arterial beds, the validity 
of our explanation awaits further experimental tests. It should be noted, however, that the 
angiotensin I converting enzyme activity of the femoral artery is lower than that of the 
coeliac artery which supplies the upper abdominal viscera (14). In the preceding paragraph 
it was suggested that E-series prostaglandins released by bradykinin might also contribute 
to the potentiating effect of SQ 14,225. If such is indeed the case, the difference in release 
of E-series prostaglandins may also explain the dissimilarity in the potentiating effect of SQ 
14,225 on the algogenic action of bradykinin.

The present results also suggest that SQ 14,225 may potentiate pathological pain in 
acute inflammation since the involvement of kinins (including bradykinin) has been suggested 
(15), and if so augmentation of the algogenic action by SQ 14,225 would be less in the somatic 
area than in the visceral area. Alternatively, if SQ 14,225 fails in this regard, the involve 
m ent of kinins in pathological pain in acute inflammation should be ruled out.

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