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Pharmacological investigation on nigrescigenin–a cardenolide from *Parquetina nigrescens* (Afzel.) Bullock: comparative studies on cardiotonic effects of *Parquetina nigrescens*, g-strophanthin and noradrenaline in guinea-pig isolated atria

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Abstract

The cardiotonic and catecholamine-like effects of *Parquetina nigrescens* extract-induced contractile force of guinea-pig left and right atria were investigated in-vitro. Isometric contractions were recorded. *P. nigrescens* extract, 5–150 μg mL^{-1}, increased the force of contraction dose dependently in electrically driven left atria. The concentration of *P. nigrescens* extract producing 50% of the maximal effect (EC_{50} value) was 7.5 μg mL^{-1}. The positive inotropic response differed from that of g-strophanthin by its high rate of onset and its complete reversibility upon removal of the extract from the incubation medium. In spontaneously beating right atrial muscle, *P. nigrescens* extract increased the rate of contractions. Its positive chronotropic and inotropic effects were partly antagonized by propranolol and atenolol indicating the presence of an adrenergic acting principle in *P. nigrescens* extract. In contrast, the inotropic response to *P. nigrescens* extract could not be completely suppressed by β-blocking agents, suggesting that the force of contraction is not only increased by a sympathomimetic ingredient of *P. nigrescens* extract but also by the cardenolides known to be present in *P. nigrescens*.

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Introduction

Parquetina nigrescens (Afzel.) Bullock (Periplocaceae) has been extensively used in traditional medicine in West Africa to treat a variety of disorders (Angenot 1934; Kerharo & Bouquet 1950; Watt & Breyer-Brandwijk 1962; Bouquet 1967, 1972; Ogundaini & Okafor 1987). In East region of Ivory Coast, an aqueous extract of the leaves is prescribed as an oral anti-asthma remedy by Abron traditional therapists. Previous studies showed the existence of cardenolides in *P. nigrescens*. The active components isolated from the leaves, seeds and roots were nigrescigenin, periplogenin and g-strophanthin (Schenker et al 1954; Mauli & Tamm 1957; Berthold et al 1965a, b; Brandt et al 1966; Marks et al 1975). Pharmacological investigations indicate that leaf extract of *P. nigrescens* (*P. nigrescens* extract) exerts an increase of the contractile response in pregnant rat isolated myometrium smooth muscle (Datté et al 1996). Moreover, the aqueous extract of the plant has a stimulant effect in guinea-pig isolated auricles (Datté & Ziegler 1998; Datté et al 1999).

Despite continuing advances in understanding of the basic pharmacology of the cardiac glycosides, digitalis intoxication remains a common clinical problem. This

prompts the search for new, similar, drugs from natural sources, which increase cardiac muscle contractility but that have a large therapeutic index. In this study, we sought to investigate, by use of available pharmacological tools, the effects of *P. nigrescens* extract on atria isolated from guinea-pig. Furthermore, experiments were conducted to characterize the pharmacological mechanisms underlying the similarities of *P. nigrescens* extract to a classic cardiac glycoside, g-strophanthin.

Materials and Methods

Plant material

Parquetina nigrescens (Afz.) Bullock (Bullock 1963) (Periplocaceae), collected in August 1998 from farms specialized in growing plants for medicinal purposes, was identified and authenticated by Dr Aké Assi Laurent (Department of Botany, Cocody University, Abidjan, Côte d'Ivoire). Voucher specimens (00104) were preserved and catalogued in the Herbarium of the Centre National de Floristique (Abidjan, Côte d'Ivoire).

Preparation of extract

The extract was prepared from leaves of *Parquetina nigrescens*. Leaves (3 kg) were dried at room temperature and *P. nigrescens* extract was extracted following established procedures (Datté et al 1996). The leaves were powdered and 50 g of the fine powder was macerated for 24 h in n-hexane to remove chlorophyll. The remainder was dried and extracted by vigorously shaking in distilled water for 24 h. After filtration and evaporation, the 50 mg of final extract yielded was stored at 4°C. A stock solution was obtained by dissolving small samples of the extract in water.

Drugs

g-Strophanthin, propranolol (hydrochloride), atenolol and verapamil were obtained from Sigma Chemical Company (St Louis, MO), and noradrenaline from Hoechst A. G. (Frankfurt am Main, Germany). Stock solutions (10^{-2} M) were prepared daily. All drugs were dissolved in water. Further dilution was made with distilled water for all drugs.

Animals

Guinea-pigs, 350–400 g, were obtained from the Animal House of Christian-Albrechts University (Kiel, Germany). All the guinea-pigs were housed at constant temperature (25°C), with a 12-h light–dark photoperiod and 60% relative humidity. Standard laboratory chow was used.

Guinea-pig isolated atria preparations

The guinea-pigs were sacrificed by cervical dislocation. Atria were immediately removed, and cleaned from fat and connective tissues. The preparations were immediately placed into Tyrode solution containing (mM): NaCl, 148.9; KCl, 2.7; $CaCl_2$,1.8; $NaPO_4H_2$, 0.2; $NaCO_3H$, 11.9; $MgCl_2$, 1.2; and glucose, 5.5.

Experimental protocol

The preparations were mounted individually in an organ bath filled with Tyrode solution (20 mL) maintained at 35°C and continuously aerated with 95% O_2 and 5% CO_2 (pH 7.4). Left atria preparations were paced electrically by two platinum electrodes (frequency, 3 Hz; impulse of duration, 5 ms). Right atria preparations were spontaneously beating. Isometric contractions were recorded with a chart recorder Hellige (Freiburg, Germany) via a strain gauge (Swema, Stockholm, Sweden). A pre-load of 1 g was applied. After a 10-min equilibration period, drug was directly introduced into the organ bath and the magnitude of the contractions (inotropic effect) was evaluated in both left and right atrial muscles. The rate of contractions (chronotropic action) was obtained in spontaneously beating guinea-pig right atria.

A second concentration–effect curve to *P. nigrescens* extract was then constructed in the continued presence of antagonist. All antagonists were evaluated at a minimum of two concentrations, each of which was tested in separate tissues. After construction of the first concentration–effect curve to *P. nigrescens* extract, tissues were washed with Tyrode solution for 10 min.

The concentration–response curves for *P. nigrescens* extract, g-strophanthin or noradrenaline on spontaneous and stimulated contractions were obtained by increasing the concentrations. Either propranolol or atenolol were incubated in the organ bath for 15 min before the others drugs were added to the solution. Inhibition of the *P. nigrescens* extract response was evaluated by comparing the mean contractions obtained before and after the exposure of the tissue to adrenoceptor-antagonist compounds.

Statistical analysis

Results are expressed as means $\pm$ s.e.m. obtained from (n) separate experiments. Statistical analysis of the data

between curves obtained in separate preparations was performed by unpaired Student's *t*-test. A P value smaller than 0.05 was considered to be statistically significant. The maximal effect of the drug (E_{max}) and the concentration producing 50% of the maximal effect (EC_{50} value) were determined graphically and calculated by non-linear regression. The curves were fitted to the individual response data by computer program (GraphPad Prism Software, San Diego, CA).

Results

Contractile responses to *P. nigrescens* extract of guinea-pig atria preparations: effects of *P. nigrescens* extract in comparison with g-Strophanthin

The contractile force of preparations under the experimental conditions amounted to 5.6 ± 0.5 mN. *P. nigrescens* extract, 5–150 μg mL^{-1}, enhanced the contractile force in electrically driven left atria and spontaneously beating right atria of guinea-pigs, causing a graded increase in the rate and force of contraction in a dose-dependent manner ($EC_{50} = 7.5$ μg mL^{-1}; Figure 1A, data for right atria not shown). g-Strophanthin (1×10^{-9} M to 5×10^{-7} M) also concentration-dependently increased the contractile response. In contrast, concentrations $> 3 \times 10^{-6}$ M were necessary to observe a significant negative inotropic effect of g-strophanthin. Finally, a biphasic dose–response curve for g-strophanthin was obtained (Figure 1B).

The time-to-peak tension response to *P. nigrescens* extract (150 μg mL^{-1}) was 10 ± 3 min. The organ reperfusion during 5–7 min with Tyrode solution showed contractions as in control conditions. No arrhythmia was observed. However, the time-to-peak tension response to g-strophanthin (5×10^{-6} M) was 25 ± 5 min, and these effects were irreversible.

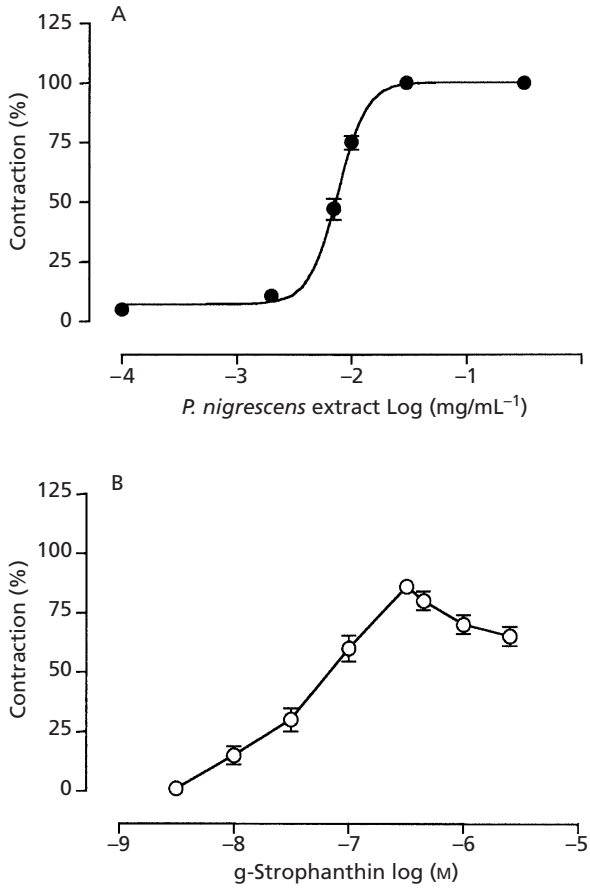

Figure 1 Concentration–response curves for (A) *P. nigrescens* extract and (B) g-strophanthin in electrically stimulated twitch contraction of guinea-pig left atria. Positive inotropic responses are expressed as percentage of the maximal response to *P. nigrescens* extract or to g-strophanthin. Values are means ± s.e.m. (n = 8 for *P. nigrescens* extract, n = 6 for g-strophanthin; $*P < 0.05$).

Frequency-dependence of *P. nigrescens* extract action: magnitude of *P. nigrescens* extract effect on the contractile activity in the guinea-pig atria preparations

We have carried out some classic experiments as described by Benforado (1958), Busse et al (1979) and Herzig et al (1995) in which the cardenolides effects were influenced by stimulation frequency. The effects of *P. nigrescens* extract (20 μg mL^{-1}) on the time to peak tension (TtPT) and the time to 90% contraction (KD 90%) were evaluated. The latent time for obtaining the maximum response was shortened when frequency was increased. There was a bell-shaped relationship observed between frequency and contractile response to *P. nigrescens* extract with a maximum value at 2.2 Hz. Increasing the frequency over the range 0.1–2 Hz increased the contractile force of guinea-pig isolated atria. But at high frequency (up 2.5 Hz), a long period was needed to reach the maximum response to *P. nigrescens* extract (Figures 2A and 2B).

Both *P. nigrescens* extract (150 μg mL^{-1}) and noradrenaline (5×10^{-7} M) induced an increase of the rate and force of contractions in spontaneously beating right atria and reached maximal tension at 45 s and 48.3 s, respectively. However, the time to reach the maximal

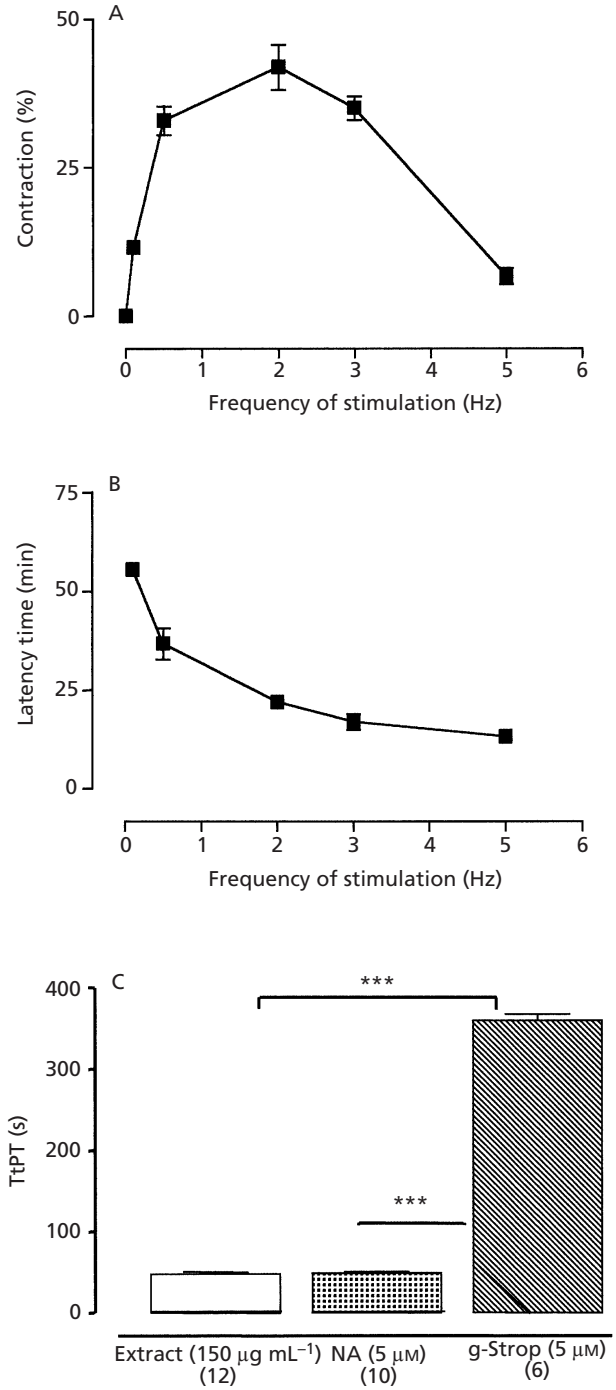

Figure 2 Frequency-dependence of *P. nigrescens* extract action (A) and latent period (B) in spontaneous beating guinea-pig right atria. Positive inotropic response is expressed as percentage of the maximal response to *P. nigrescens* extract (20 μg mL^{-1}) C. Time to peak tension (TtPT) in the presence of *P. nigrescens* extract (150 μg mL^{-1}; n = 12), noradrenaline (NA, 5×10^{-7} M; n = 10) and g-strophanthin (g-Strop, 5×10^{-7} M; n = 6). Values are means ± s.e.m, (***$P < 0.01$). The value written under each histogram is (n) separate experiments.

tension with g-strophanthin (5×10^{-7} M) was 180.8 s (4-fold, Figure 2C). The original tracings illustrating the inotropic and chronotropic effects of *P. nigrescens* extract and noradrenaline are shown in Figures 3A and 3B, respectively.

Inhibitory effects of beta adrenoceptors on *P. nigrescens* extract-induced contractile responses in guinea-pig isolated atria preparations

The concentration–response curve for *P. nigrescens* extract was shifted to the right after propranolol pre-treatment; the half-maximum contraction was achieved at $-(\log EC_{50})$ 12 (EC_{50} value: 12 μg mL^{-1}) and the E_{max} contraction was lowered to 64.5% (Figures 3A). The antagonism was reversible and there was a non-parallel shift to the right. However, after propranolol pre-treatment, no significant rightward shift of the concentration–response curve for g-strophanthin (1×10^{-9} M to 1×10^{-7} M) was observed (Figures 3B). The EC_{50} and E_{max} values for g-strophanthin were not affected by propranolol pre-treatment.

Figures 3C shows the time-course curves of contraction response to *P. nigrescens* extract (150 μg mL^{-1}) in spontaneously beating right atria of guinea-pig in the presence of propranolol (1×10^{-7} M and 1×10^{-6} M). *P. nigrescens* extract's positive chronotropic effect was partly inhibited by propranolol. The displacement of the time-course curves for *P. nigrescens* extract by propranolol was dose dependent. The effect of *P. nigrescens* extract was compared with that of noradrenaline. Noradrenaline's positive chronotropic effect in isolated right atria was affected by propranolol at the same concentrations (Figures 3D), whereas the effect of g-strophanthin was not affected by propranolol pre-treatment.

Inhibitory effects of atenolol on *P. nigrescens* extract-induced contractile responses in guinea-pig isolated atria preparations

Figure 4A illustrates the time-course curves of the positive chronotropic effect induced by *P. nigrescens* extract at 150 μg mL^{-1} in spontaneously beating isolated right atria of guinea-pig. The displacement of the time-course curves for *P. nigrescens* extract by atenolol was dose dependent. The inhibition induced by atenolol was reversible. Similar results were obtained with noradrenaline. The time-course curve for noradrenaline (5×10^{-7} M) was antagonized by atenolol dose depen-

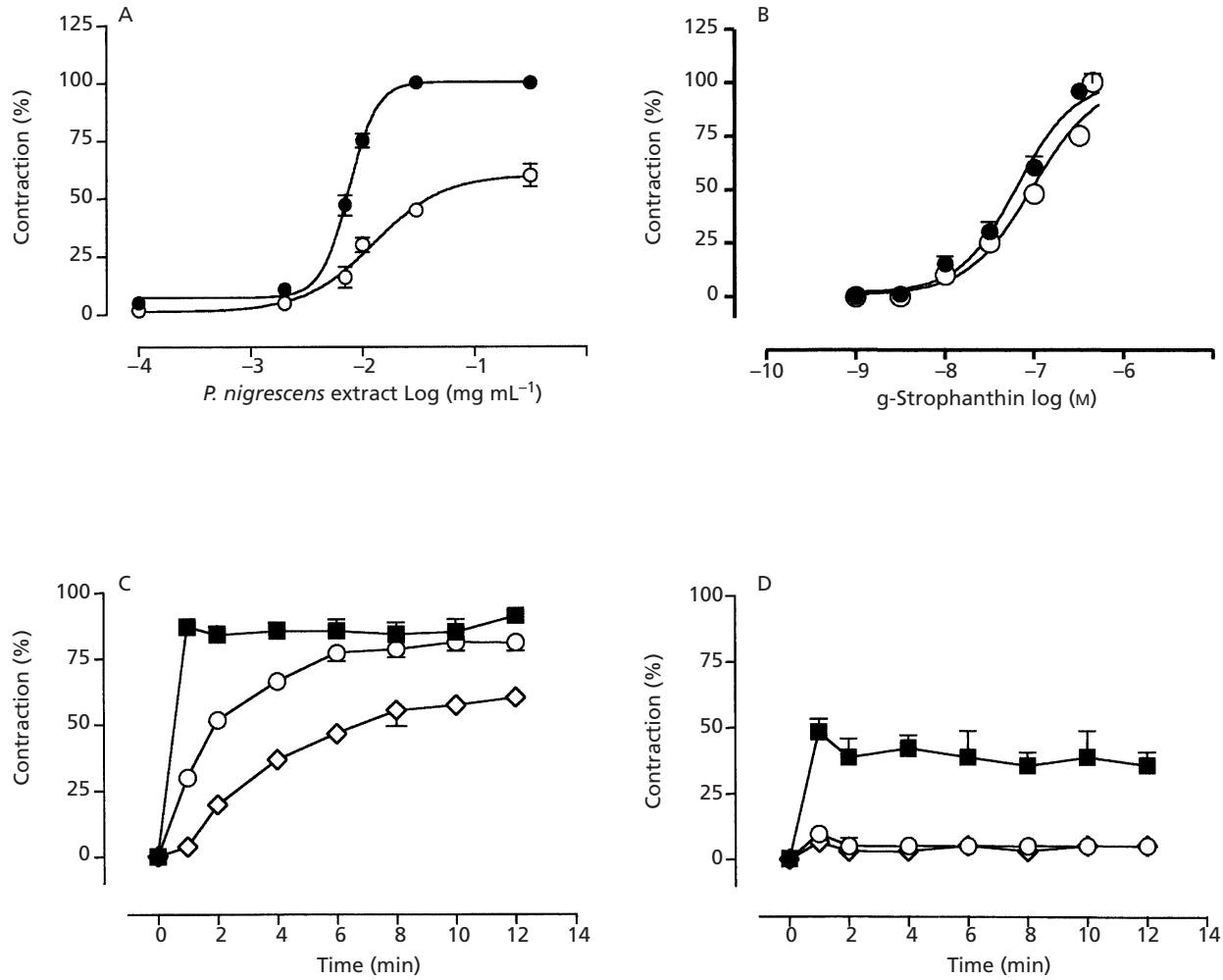

Figure 3 Concentration-dependent response of electrically stimulated twitch contraction of guinea-pig isolated left atria induced by *P. nigrescens* extract (A) and g-strophanthin (B) in the absence (●) or presence of propranolol 10^{-7} M (○). Response is expressed as a percentage of the maximal response to *P. nigrescens* extract. Values are means ± s.e.m., n = 6 for *P. nigrescens* extract, n = 5 for g-strophanthin; $*P < 0.05$. Also shown is the time-course response curves of right atria for *P. nigrescens* extract (C) and for noradrenaline (D) in the absence (■) or presence of propranolol 10^{-8} M (○) or 10^{-7} M (□). Response is expressed as percentage of the maximal response to *P. nigrescens* extract. Values are means ± s.e.m., n = 6 for *P. nigrescens* extract, n = 5 for noradrenaline; $*P < 0.05$.

dently (Figure 4B). However, the time-course curve for g-strophanthin was not affected by atenolol pre-treatment (data not shown).

Discussion

Characterization of glycoside-like effects of *P. nigrescens* extract in guinea-pig isolated atria preparations

The heart glycosides are used clinically for their therapeutic benefit in heart failure (Baumgarten 1963; Smith 1975; Bentfeld et al 1977; Busse et al 1979; Morgan & Morgan 1984). Treatment with cardiac glycosides elicits concentration-dependent positive inotropic effects on isolated myocardial tissue (Coraboeuf et al 1953; Brauwald et al 1961; Akera 1977; Bentfeld et al 1977). *P. nigrescens* was chosen for investigation since some cardenolides (e.g., nigrescigenin) were isolated by Berthold et al (1965b) and Brandt et al (1966).

Our data demonstrate clearly that *P. nigrescens* extract, as well as noradrenaline, significantly enhanced the contractile force of guinea-pig isolated atria in a concentration-dependent manner. In contrast, the dose-response curve for g-strophanthin was biphasic. Similar results were obtained by Kenakin & Boselli (1991) in rat

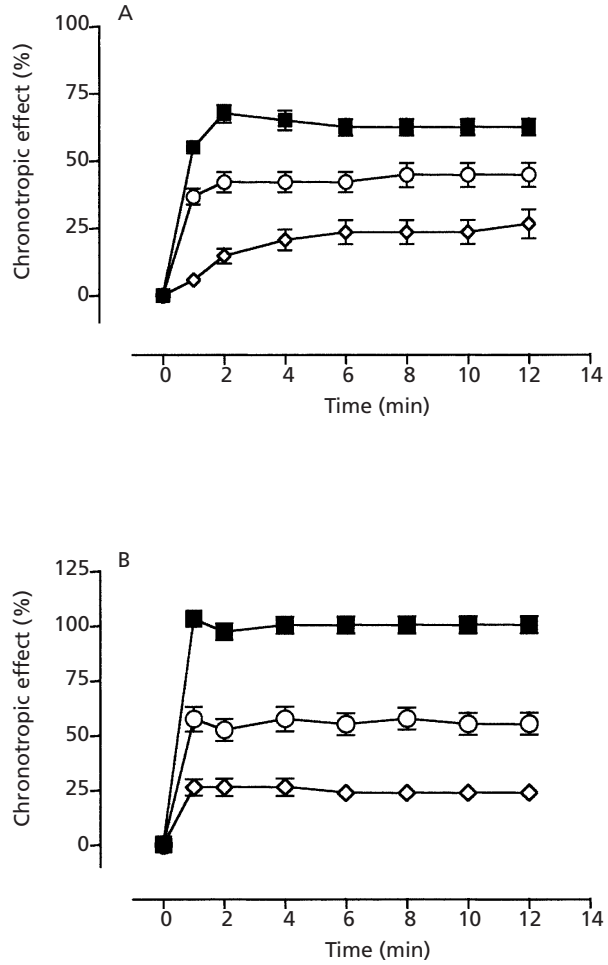

Figure 4 Time-course response curves of guinea-pig isolated right atria for *P. nigrescens* extract (A) and for noradrenaline (B) in the absence (■) or presence of atenolol 10^{-8} M (○) or 10^{-7} M (□). Response is expressed as a percentage of the maximal response to *P. nigrescens* extract. Values are means ± s.e.m.; n = 5 for *P. nigrescens* extract; n = 5 for noradrenaline, $^{*}P < 0.05$.

atria. *P. nigrescens* extract's positive inotropic effect was very fast, as was that of noradrenaline, but was not like that of classic cardiac glycosides which needed more time to reach the maximum positive inotropic effect. Moreover, the frequency-dependent action of *P. nigrescens* extract showed a bell-shaped curve, like those reported by Benforado (1958), Kenakin et al (1991) and Herzig (1984) for classic heart glycosides. The findings reported in this study confirm previous observations that *P. nigrescens* extract contains cardiac glycosides (Datté et al 1996). g-Strophanthin, however, did not induce a positive chronotropic effect, as did *P. nigrescens* extract or noradrenaline. Tauchert & Loew (1995) carried out similar investigations with *Crataegi folium* cum. for treatment of heart failure, which, like the catecholamines, induced a positive chronotropic action.

To investigate adrenoceptor activation by *P. nigrescens* extract, propranolol, which antagonizes the cardiac effect of sympathomimetics in a competitive manner, was used as a pharmacological tool (Black et al 1964; Nakano & Sakato 1966; Mattsson et al 1983; Herzig et al 1995). Our results show an obvious parallelism of the sympathetic amine positive inotropic effect and *P. nigrescens* extract's action in guinea-pig isolated atria (i.e. a fast inotropic effect, positive chronotropic and reversible inotropic actions). Therefore, we attempted to classify the receptor responsible by the use of selective beta-adrenoceptor-blocking agents.

Pharmacological characterization of the contractile responses to *P. nigrescens* extract

The effects of *P. nigrescens* extract on guinea-pig isolated atria were blocked by beta-adrenoceptor-blocking agents. However, adrenoceptor antagonists did not influence the g-strophanthin-induced contractile responses. Similar observations were demonstrated by Lüllmann & Peters (1979) and Peters (1982), who reported that the classic cardiac glycoside g-strophanthin induced a positive inotropic effect in guinea-pig atria that was unaffected by prior treatment with propranolol. The guinea-pig isolated myocardium is used to assay beta adrenoceptors, and is known to contain heterogeneous populations of these receptors (Grobecker & Krämer 1996). Radioligand binding studies have demonstrated that most guinea-pig heart beta adrenoceptors are of the β_1-subtype ($\sim$ 80%), while a minority is of the β_2-subtype (Hedberg et al 1980).

Our data suggest that the ability of *P. nigrescens* extract to produce a positive inotropic effect may be also mediated by beta adrenoceptors.

These results indicate that *P. nigrescens* extract did not act with the mechanism of classic heart glycosides, suggesting that *P. nigrescens* extract could contain a second group of compounds which act like catecholamines, as reported in previous studies in isolated smooth muscles (Datté & Ziegler 1998; Datté et al 1999).

P. nigrescens extract has a potent cardiotonic effect. In contrast to the positive chronotropic effect, the inotropic response to *P. nigrescens* extract could not be completely suppressed by the beta-blocking agent, suggesting that the force of contraction is not only increased by a sympathomimetic ingredient of *P. nigrescens* extract but also by the cardenolides (e.g., nigrescigenin and periplogenin) known to be present in *P. nigrescens*

(Berthold et al 1965b; Brandt et al 1966; Marks et al 1975).

Conclusion

In conclusion, the *P. nigrescens* extract effects in guinea-pig isolated atria could be characterized by the following properties:

P. nigrescens extract exerted pronounced positive inotropic effects, as did noradrenaline and g-strophanthin.

The *P. nigrescens* extract-induced contractile force was partly blocked by propranolol. However, propranolol or atenolol completely inhibited the noradrenaline-induced inotropic effect.

The similarities of *P. nigrescens* extract, g-strophanthin, and noradrenaline demonstrated that *P. nigrescens* extract induces a contractile response by inhibition of Na^+-K^+-ATPase as well as by activation of beta adrenoceptors. *P. nigrescens* extract has potent positive inotropic effects.

References

Akera, T. (1977) Membrane adenosinetriphosphatase: a digitalis receptor? *Science* **198**: 569–574

Angenot, L. (1934) Les poisons de flèches africaines. 1. Zaire, Rwanda et Burundi. *Africa Tervuren* **8**: 72–74

Baumgarten, G. (1963) *Die herzwirksamen Glykoside*. Edition, Leipzig, pp 1–9

Benforado, J. M. (1958) Frequency-dependent pharmacological and physiological effects on the rat ventricle strip. *J. Pharmacol. Exp. Ther.* **122**: 86–100

Bentfeld, M., Lüllmann, H., Peters, T., Proppe, D. (1977) Interdependence of ion transport and the action of ouabain in heart muscle. *Br. J. Pharmacol.* **61**: 19–27

Berthold, R., Wehrli, W., Reichstein, T. (1965a) Die Glykoside von *Parquetina nigrescens* (Afz.) Bullock. *Helv. Chim. Acta* **48**: 1634–1658

Berthold, R., Wehrli, W., Reichstein, T. (1965b) Die Glykoside von *Parquetina nigrescens* (Afz.) Bullock. *Helv. Chim. Acta* **48**: 1659–1665

Black, J. W., Crowther, A. F., Shanks, R. G., Smith, L. H., Dornhorst, A. (1964) A new adrenergic β-receptor antagonist. *Lancet* **i**: 1080

Bouquet, A. (1967) Note sur la préparation du poison de flèches dans le Nord-Congo (Brazzaville). *J. Agric. Trop. Bot. Appl.* **14**: 359–361

Bouquet, A. (1972) *Plantes Médicales du Congo-Brazzaville. Uvariopsis, Pauridiantha, Fiospyros*. Travaux et Documents de l'ORSTOM, 13

Brandt, R., Kaufmann, H., Reichstein, T. (1966) Nigrescigenin, Identifizierung mit Sarmentosigenin. *Helv Chim Acta* **25**: 1844–1849

Brauwald, E., Bloodwell, R. D., Goldberg, L. I., Morrow, A. G. (1961) Studies on digitalis. IV: Observations in man on the effects of digitalis preparations on the contractility of the non-failing heart and on total vascular resistance. *J. Clin. Invest.* **40**: 52–59

Bullock, A. (1963) Notes on African Asclepiadaceae IX. *Kew Bulletin* **15**: 193–206

Busse, F., Lüllman, H., Peters, T. (1979) Concentration dependence of the binding of ouabain to isolated guinea pig atria. *J. Cardiovasc. Pharmacol.* **1**: 687–698

Coraboeuf, E., de Loze, C., Boistel, J. (1953) Action de la digitale sur les potentiels de membrane et d'action du tissu conducteur du cœur de chien étudiée à l'aide de micro-électrodes intracellulaires. *C. R. Soc. Biol.* **143**: 1329–1331

Datté, J. Y., Ziegler, A. (1998) *Parquetina nigrescens* contains an indirect sympathomimetic. (Abstract) *Naunyn Schmiedebergs Arch. Pharmacol.* **357**: R27

Datté, J. Y., Offoumou, A. M., Manda, O. M. (1996) Uterotonic effects of hydromethanolic extract of *Parquetina nigrescens* (Periplocaceae) on spontaneous contractile activity in the isolated myometrium of pregnant rats. *J. Ethnopharmacol.* **53**: 15–20

Datté, J. Y., Ziegler, A., Tillement, J. P. (1999) Sympathomimetic effects of *Parquetina nigrescens* Afz. (Periplocaceae) extract in isolated portal vein smooth muscle. *Gen. Pharmacol.* **32**: 551–556

Grobecker, H. F., Krämer, B. K. (1996) *Beta-Rezeptoren Antagonisten–Pharmakologie und therapeutische Anwendung*. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, pp 25–39

Hedberg, A., Kempf, F., Josephson, M. E., Milinoff, P. B. (1980) Coexistence of beta-1 and beta-2 adrenergic receptors in the human heart: effects of treatment with receptor antagonists or calcium entry blockers. *J. Pharmacol. Exp. Ther.* **234**: 561–568

Herzig, S. (1984) *Über den Einfluss von Herzglykosiden und von Ca^{2+} auf die mechanische Aktivität der Herzmuskulatur von Ratten*. Dissertation, Christian - Albrechts - Universität zu Kiel

Herzig, S., Jischa J., Beinhauer, A., Geirhos, B., Tacke, K., Hempelmann, R. G. (1995) Quinidine-induced potentiation of cardiovascular effects of nitrendipine: functional aspects and possible molecular mechanisms. *Naunyn Schmiedebergs Arch. Pharmacol.* **351**: 636–643

Kenakin, T. P., Boselli, C. (1991) Biphasic dose-response curves to arecoline in rat atria: mediation by a single promiscuous receptor or two receptor subtypes? *Naunyn Schmiedebergs Arch. Pharmacol.* **344**: 201–205

Kenakin, T. P., Ambrose, J. R., Irving, P. E. (1991) The relative efficiency of beta adrenoceptor coupling to myocardial inotropy and diastolic relaxation: organ-selective treatment for diastolic dysfunction. *J. Pharmacol. Exp. Ther.* **257**: 1189–1197

Kerharo, J., Bouquet, A. (1950) *Plantes Médicinales et Toxiques de la Côte d'Ivoire-Haute Volta*. Vigot Frères Edition, Paris, 10

Lüllmann, H., Peters, T. (1979) Action of cardiac glycosides on the excitation-contraction coupling in heart muscle. *Progr. Pharmacol.* **2**: 1–54

Marks, W. H., Fong, H. H. S., Tin-Wa, M., Farnsworth, N. R. (1975) Cytotoxic principles of *Parquetina nigrescens* (Afz.) Bullock (Asclepiadaceae). *J. Pharm. Sci.* **24**: 1674-1677

Mattsson, H., Hedberg, A., Carlsson, E. (1983) Intrinsic sympathomimetic activity of the partial agonist prenalterol in relation to beta adrenoceptor interaction in various tissues *in vitro*. *J. Pharmacol. Exp. Ther.* **224**: 654–661

Mauli, R., Tamm, C. (1957) Die Glykoside von *Periploca nigrescens* Afz. *Helv. Chim. Acta* **40**: 299–305

Morgan, J. P., Morgan, K. G. (1984) Calcium and cardiovascular function. *Am. J. Med.* **142**: 33–46

Nakano, J., Sakato, M. (1966) Competitive antagonism between norepinephrine and propranolol. *Experientia* **12**: 112

Ogundaini, A. O., Okafor, E. O. (1987) Isorhoifolin, a flavonoid glycoside from *Periploca nigrescens* leaves. *Planta Medica* **54**: 391

Peters, T. (1982) Dependence of the dose-response relationship for cardiac glycosides on the experimental conditions. *Naunyn-Schmiedebergs Arch. Pharmacol.* **319**: R43

Schenker, E., Hunger, A., Reichtein, T. (1954) Die Glykoside von *Periploca nigrescens* Afzel. *Helv Chim Acta* **37**: 1004–1036

Smith, T. W. (1975) Digitalis toxicity: epidemiology and clinical use of serum concentration measurements. *Am. J. Med.* **58**: 470–476

Tauchert, M., Loew, D. (1995) *Crataegi folium* cum bei Herzinsuffizienz, Klinische Studien mit normierten *Crataegus*-Extrakten. In: Loew, D., Rietbrock, N. *Phytopharmaka in Forschung und Klinischer Anwendung*. Darmstadt, Germany, pp 137–144

Watt, J. M., Breyer-Brandwijk, M. G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd edn, Livingstone, Edinburgh and London, pp 562–564