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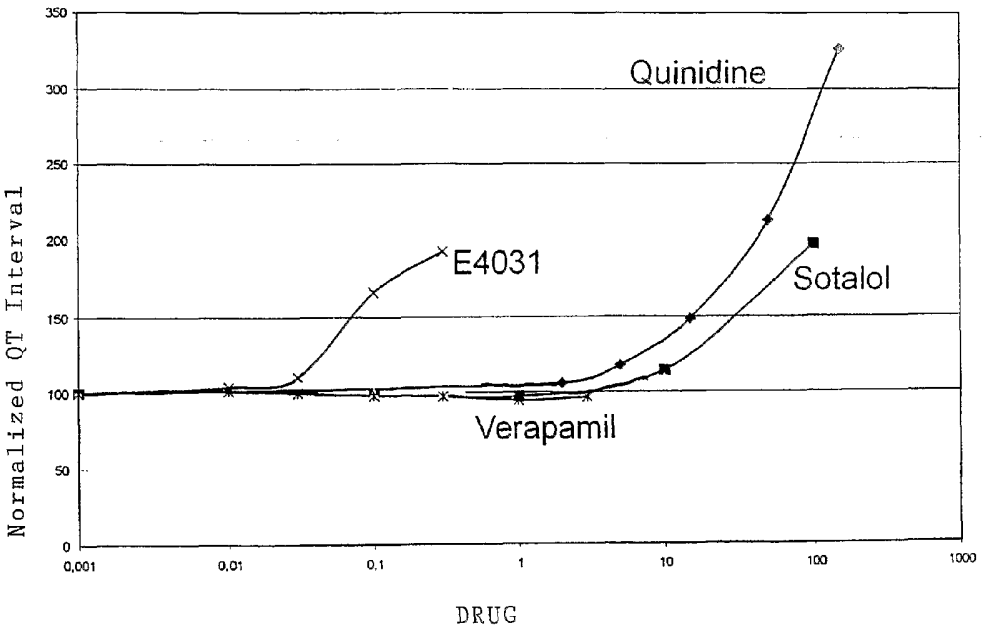
(54) Title: METHOD FOR DETERMINING THE INFLUENCE OF A TEST SUBSTANCE ON THE HEART ACTIVITY OF A VERTEBRATE



(57) Abstract: A method for determining the influence of a test substance on the heart activity of a vertebrate is proposed which comprises the following steps: a) preparation of a culture of spontaneously active heart cells of the vertebrate, b) extracellular measurement of electrophysiological data of the heart cells from step a), c) addition of the test substance to the culture from step a), d) repetition of the measurement from step b), and e) comparison of data from step b) and step d).

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Method for determining the influence of a test substance on the heart activity of a vertebrate

The present invention relates to a method for determining the influence of a test substance on the heart activity of a vertebrate, in particular on the interval between ventricular depolarization and repolarization.

It has long been known that many substances with which animals and humans come into contact may have an influence on heart activity. These substances can generally be environmental factors which are absorbed by breathing or through the skin or in food, or intentionally administered pharmaceutical substances and cosmetic products. In the context of the present application, these substances are generally referred to as "substances" or "test substances".

To protect livestock animals, domestic animals and, in particular, human beings from the harmful or damaging influence of such substances, not only are the toxicity and the desired action of these substances tested, but increasingly also their undesired influence on heart activity, before said substances are released for use.

In addition, however, there is also a need to test substances already in use, in particular approved medicaments or those being used in clinical trials, in order to ascertain their influence on heart activity. In the case of medicines in particular, undesired side effects on heart rate activity often arise which are not detected in the context of the clinical trials conducted for approval of these medicines or in the preliminary stages of such clinical trials. This leads to a potential danger to persons participating in clinical studies in particular, and to all patients in general.

Such undesired side effects may only become apparent as harmful for example after prolonged administration, in combination with other substances, or in the presence of risk factors, for example hypokalemia, or structural heart disease, so that they cannot be reliably detected either in the preliminary phases of or during the conduct of clinical trials and studies.

In the context of clinical trials and studies, the influence of substances on heart activity can be determined by what is called electrocardiography, that is to say by recording the ventricular stimulus conduction in the heart in the course of heart activity, the time course being plotted as a tracing, a so-called electrocardiogram. The tracings represent the sum of the intensities and directions of the electrical potentials in the individual myocardial and nerve fibres, and they are recorded by leads assigned in different spatial configurations with respect to the heart, for example on the extremities.

An electrocardiogram is divided into different elements which can be assigned to different physiological processes. The P

wave records the excitation of the atrium, and it is followed by the isoelectric PQ segment which records the conduction time of the excitation to the ventricles. The QRS complex corresponds to the ventricular excitation; this is followed by the ST segment, which in turn is followed by the T wave which corresponds to the ventricular repolarization. From the changes in the segment duration and the shape of the elements in the recorded tracings, it is possible to draw important conclusions regarding the heart.

An important variable here is the QT interval, that is to say the time from the start of the QRS complex to the end of the T wave, reflecting the overall electrical action of the ventricle. This QT interval is therefore a measure of the duration of the ventricular depolarization and repolarization.

It is known that a change in the QT interval leads to increased health risks, particularly as a result of rhythm disturbances. For example, a prolongation of the QT interval leads to an increased risk of ventricular tachycardia and fatal arrhythmia. This change in the QT interval may be congenital or may have been acquired as a result of disease or ingestion of harmful substances.

Numerous medicaments are known with which a desired change in the QT interval can be effected in order to counteract pathological changes. On the other hand, there are many substances which have an undesired influence on the QT interval which in many cases goes unrecognized or cannot be detected in conventional clinical studies.

However, even if the influence of substances on heart activity and the associated side effects, for example QT prolongation, could be reliably and conclusively determined with the aid of electrocardiography in the context of clinical studies, the conduct of such studies nevertheless constitutes an unacceptable risk to those participating in these studies and investigations. Quite apart from the ethical problems surrounding the conduct of tests on animals, these tests in the final analysis are not purposeful because there is a limit to the extent to which data from animal tests can be transposed to humans.

In addition, not only would carrying out clinical studies or animal experiments to test the many substances already on the market for their influence on heart activity be associated with high costs, it would also take a considerable time before each particular study was concluded.

In view of the above, one object of the present invention is to make available a method of the aforementioned type which can be performed quickly and inexpensively and without the need for tests on humans or animals.

According to the invention, this object is achieved by a method of the aforementioned type which comprises the following steps:

a) preparation of a culture of spontaneously active heart cells of the vertebrate,

b) extracellular measurement of electrophysiological data of the heart cells from step a),

c) addition of the test substance to the culture from step a),

d) repetition of the measurement from step b), and

e) comparison of data from step b) and step d).

The object of the invention is achieved in full in this way.

The inventors of the present application have in fact found that electrophysiological data from extracellular recordings from cultured heart cells permit conclusions on heart activity which conventionally can be obtained only from an ECG lead.

This finding is all the more astonishing given that, for example, ventricular repolarization, which crucially defines heart activity, is a complex physiological process. It is the result of the interaction of many ion channels and transporters whose activities under physiological conditions are very much dependent on one another and are determined, for example, by intracellular and extracellular ion concentrations, membrane potential, heart rate, metabolism, etc.

Against this background, it was not to be expected that *in vitro* measurements can be brought into any reliable correlation with the complex *in vivo* conditions and permit conclusions regarding the influence of substances on heart activity.

However, the inventors have not taken what is, despite the doubtful correlation, the perhaps obvious course of determining the action potential of heart cells by intracellular measure-

ments and drawing conclusions from these about heart activity. Instead, they have been able to show that extracellular measurements of the field potential of heart cells in culture provide evidence regarding the *in vivo* conditions.

This evidence is of an essentially qualitative nature and indicates whether and if so what influence the tested substance has on heart activity, particularly on the QT interval. It therefore represents a kind of pre-screening for determining whether the test substance has to be further evaluated if appropriate in clinical studies or whether it is harmless with respect to heart activity. The qualitative evidence on whether a QT prolongation or shortening is to be reckoned with also provides important information on the design and evaluation of clinical studies and on the nature of the side effects that may be expected, particularly also in connection with previous diseases or existing medication. By means of an *in vitro* test which can be performed easily, quickly and inexpensively compared to human or animal experiments, the safety of test subjects and patients can thus be significantly increased.

The measurements can be carried out on individual heart cells, on aggregates of heart cells or on tissue composites or aggregates, and the cells can be native heart cells, for example from biopsy material or from clinical material, or, alternatively, cell lines can also be used. The important point is merely that the culture contains spontaneously beating heart cells or similar, for example cells derived from stem cells.

In this way it is possible to draw general conclusions concerning the influence of the test substance on heart activity in

general and also specific conclusions concerning the influence in individual patients. Thus, it is possible not just to evaluate the respective substance in general, but also to investigate an individual patient to determine how he reacts to certain substances, so that the method according to the invention can be employed also in the context of a diagnostic procedure or in the run-up to a therapeutic procedure.

The method can also be used to select or produce a specifically tailored medicament for a specific patient, taking into account his physiological status and his particular disease. To do so, heart cells from the patient himself are used so that it is possible to carry out an *in vitro* check as to how the particular patient reacts to the substance. It is thus possible to check, for example prior to a course of medication, whether a substance with known but rare side effects can be administered to a specific patient. The heart cells in this case derive either from biopsies or from surgical interventions, balloon dilations, etc., or are differentiated from the patient's stem cells.

Therefore, according to another object, the invention is directed to a method for treating a human individual or an animal, comprising the steps of:

a) selecting a test substance suitable for said treatment,

b) providing heart cells of said human individual or animal,

c) determining the influence of said test substance on the activity of said heart cells, by the method of anyone of claims 1 – 16,

d) preparing a pharmaceutical composition containing said test substance in case said influence on the heart activity is admissible.

All in all, the method according the invention can be performed inexpensively and quickly compared to clinical studies. Prior to tests on humans or animals, it permits determination of potential dangers to the subjects and a better design and better interpretation of clinical studies.

The method according to the invention thus makes it easier to determine the potential danger to and protection of subjects and patients in connection with new and with known medicines, and the investigation of environmental factors of all kinds, including in veterinary medicine or in connection with work place safety, and in the cosmetics industry. It provides information on whether a substance is to be classified as reasonably safe in respect of its influence on heart activity, in particular on the QT interval, or whether particular caution is required in administering it and whether further or special clinical studies may be necessary.

The measurements were carried out in the first instance with so-called MEAs (multi-electrode arrays), as are marketed by the Applicant, for example as the MEA60 system. In these devices, a number of microelectrodes are integrated on a substrate, and heart cells are cultured on these. The microelectrodes are used

to record the field potentials at different sites of the tissue culture, so that, in addition to the time course of the field potential at a measurement site, it was also possible to investigate the spatial propagation of the signals. Initial measurement results showed that it is not necessary to carry out measurements at different sites in a tissue culture or on individual cells in order to obtain reliable evidence concerning the time course of the field potential in the culture and to draw conclusions on the influence of the tested substance on heart activity. Rather, it is sufficient to use a measurement electrode or microelectrode which is completely covered by a cell or by an electrically coupled cell aggregate.

The measurements are therefore preferably carried out with the aid of a device for extracellular recording of electrophysiological data, as is described for example in DE 197 12 309. With this device it is possible to carry out measurements on separate individual cells or cell composites and aggregates, which are preferably present in microcuvettes of suitable dimensions arranged in a support plate. The cells or tissue composites are cultured in the microcuvettes at the bottoms of which there is in each case at least one measurement electrode with which the field potential can be measured on cells in the respective microcuvette.

It is also possible to use a type of microtitre plate with, for example, 96 cuvettes or wells in the standard grid, a measurement electrode being arranged at the bottom of each well, and a reference electrode additionally being arranged in the well. Cell composites, that is to say tissue cultures or electrically coupled aggregates of heart cells, are incubated in the wells;

they cover the measurement electrode and their field potential is recorded.

In the various microcuvettes or wells of these devices, either heart cells of different origin can be cultured in order to investigate the influence of a substance on the different heart cells, or alternatively heart cells of a single origin are cultured and the effect of a different substance is investigated in each microcuvette. Of course, a combination of the two procedures can be chosen in which both heart cells of different origin and also different substances are tested simultaneously on a support plate with a large number of microcuvettes. In addition to the test substance or substances, reference substances can also be tested, in order for example to calibrate the measurement results.

Against this background, the present invention also concerns the use of a device for extracellular recording of electrophysiological data, for example an MEA or a support plate with microcuvettes in each of which at least one measurement electrode is arranged, in the aforementioned method.

In steps b) and d), the time course of the field potential (FP) of the culture is preferably measured, and it is further preferable for at least one parameter, FPdur and/or FPrise, to be calculated from the FP time course and compared, the change in this parameter by addition of the test substance to the culture being a measure of the influence of the substance on heart activity.

In active or beating heart cells, the field potential showed a pattern recurring in the heart beat rhythm consisting of a first minimum and a last maximum, and in which it was possible for a further maximum to precede in each case the first minimum and the last maximum. The time interval FPdur between the first minimum and the last maximum proved, in initial experiments by the inventors, to be a parameter whose change as a result of addition of a test substance is comparable to the change in the QT interval arising when this substance is administered to a patient. A substance known to cause a QT prolongation increased the parameter FPdur, while another substance known to cause a QT shortening reduced FPdur.

A further relevant parameter of the FP wave shape proved to be the duration of the falling flank in the FP wave shape from the zero line to the first minimum, which is characterized by a parameter FPrise. FPrise is calculated as the duration between reaching, for example, 10% of the first minimum and reaching the first minimum. In the experience of the inventors of the present application, a prolongation or shortening of FPrise is also reflected in a prolongation or shortening of QT.

The method according to the invention can be employed, for example, in analysis laboratories as a service provided for doctors, hospitals, pharmaceutical companies, cosmetics companies, or generally in the research and manufacturing industry, in order to gain initial qualitative data on substances which are approved or are to be approved as a medicament or cosmetic product, which occur or are intended to be used as environmental factor or environmental pollutant whose individual effect on a certain subject, patient or patient type is to be

determined. In this way, it is possible, in the preliminary stages of human and animal tests, or instead of these tests, to perform an evaluation which not only facilitates the interpretation and appraisal of further studies and investigations but has a decisive input on these.

Further advantages and features will be evident from the following description and from the attached figures.

It will be appreciated that the features mentioned above, and those to be discussed below, can be used not only in the respectively cited combination but also in other combinations or in isolation, without departing from the scope of the present invention.

Illustrative embodiments of the invention are explained in more detail in the following description in which reference is made to the figures, in which:

Fig. 1A is a representation of FP wave shapes which were derived using an MEA comprising 60 microelectrodes on a culture of spontaneously beating heart cells;

Fig. 1B is an enlarged representation of two typical FP wave shapes from Fig. 1A, showing inter alia the parameters FPdur and FPrise;

Fig. 2 shows typical measured values obtained, in the test described here, for quinidine at different concentrations;

Fig. 3 is a diagrammatic comparison between ECG, action potential, potassium ion current I(Kr) and MEA field potential for ventricular myocytes from chicken embryos without addition (normal) and with addition (QT prolongation) of quinidine; and

Fig. 4 shows, by way of comparison, the normalized QT interval for four different active substances whose effect on ventricular myocytes from chicken embryos was determined with MEAs.

Example 1: Determination of FP parameters

Heart cells from mouse embryos were cultured in DMEM supplemented with 20% FCS, L-glutamine (2 mmol/L) and nonessential amino acids (all chemicals from Sigma-Aldrich). A drop of the cell suspension (10^7 cells/ml) was placed on an MEA produced by the Applicant and having 60 microelectrodes. After attachment of the cells, culture medium was added to give a final volume of 800 µl. After 1 to 3 days in culture, the cells formed a confluent monolayer of multicellular aggregates which showed spontaneous beating activities.

The FP wave shapes measured with the MEA after 4 days in culture are shown in Fig. 1A. Different FP wave shapes were recorded on different microelectrodes, of which two representative examples are shown enlarged in Fig. 1B.

The field potential shows in each case a recurring pattern in the beat rhythm with a first minimum FPmin and a last maximum FPmax, where FPmin and FPmax could in each case be preceded by

a further maximum FPpre and FPslow. The time interval between the first minimum and the last maximum is designated as FPdur.

A further parameter of the FP wave shape is the duration of the falling or declining flank in the FP wave shape from the base line to the first minimum FPmin which is characterized by a parameter FPrise. FPrise is calculated as the duration between reaching 10% of FPmin and reaching FPmin.

It was found that FPdur and FPrise are important parameters which can be calculated from the extacellularly derived FP wave shape and which change in a manner comparable to QT upon addition of QT-modifying substances.

Example 2: Influence of QT-modifying substances on FPdur

In a method comparable to Example 1, ventricular myocytes from chicken embryos were in this case cultured on the MEA. The heart muscle cells were obtained by trypsin digestion of the isolated ventricle of chicken embryos (10-12 days after fertilization). The heart was freed of blood vessels and atria. The cells were cultured in MEM medium supplemented with 10% fetal calf serum (FCS). One to two days before the measurement, the medium was replaced by standard Tyrode solution.

The heart cells were again cultured on the MEA, the derivation and recording time being 10 minutes, which in most cases proved sufficient to permit conclusions to be drawn in this control experiment concerning heart rate and stability of the QT interval.

The standard Tyrode solution was then replaced by a Tyrode solution with 5 µM of a test substance and the change in FPdur was determined. Thereafter, the concentration of the test substance was increased in logarithmic steps. A measurement was now taken every 10 minutes.

In a first test, the substances tested were quinidine and digoxin, the influence of which on QT has long been known.

Quinidine has a QT-prolonging effect and is used as an antiarrhythmic agent; see for example W. B. Campbell, "EKG of the month: QT prolongation induced by quinidine in therapeutic doses", in J. Tenn. Med. Assoc. 1982, 75(5):340-341.

By contrast, digoxin has a QT-shortening effect and is used in chronic heart insufficiency and to prevent and treat tachycardia; see, for example, Joubert et al., "A correlative study of serum digoxin levels and electrocardiographic measurements", in S. Afr. Med. 1975, 49(29):1177-.

In measurements with quinidine, 0.5% DMSO was added to the cell Tyrode since quinidine is not water-soluble. The control experiment was also carried out with DMSO here.

Initial evaluations of these experiments showed that addition of digoxin led to a decrease in FPdur and addition of quinidine led to an increase in FPdur. In these qualitative evaluations, i.e. comparison of data from the respective control experiment and the data derived from addition of the QT-modifying substance, possible changes in the heart rate were taken into account.

Fig. 2 shows typical measured values which were determined in the tests described here for quinidine at different concentrations. Fig. 2A shows a typical time course for a field potential measured on MEAs, while Fig. 2B shows field potential courses after addition of the respectively indicated quinidine concentrations for 200 seconds. A QT prolongation increasing with concentration can be clearly seen from the increase in FPdur.

In Fig. 2C, the dose-dependent prolongation is indicated as FPdur (QT) or as FPdur normalized with the heart rate (QTc). For the normalized case, the FPdur value was divided by the square root of the time span (in seconds) between two action potentials.

Fig. 3 shows the diagrammatic comparison between ECG, action potential, potassium ion current I(Kr) and MEA field potential for ventricular myocytes from chicken embryos without addition (normal) and with addition (QT prolongation) of quinidine. It can clearly be seen that the QT prolongation observed in the ECG has its correspondence not only in the measured action potential but also in the field potential, FPdur changes measured by MEAs are therefore a direct measure for QT changes.

Fig. 4 shows by comparison the normalized QT interval for four different active substances whose effect on ventricular myocytes from chicken embryos was determined with MEAs.

It has been found that verapamil (5-[N-(3,4-dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride) in the concentration

range of from 1 nM to 3 μM has only a very slight effect on QT, although it is known as an antagonist for the L-type calcium channel and blocks potassium channels. Although verapamil would therefore be ruled out as potential medicament in an HERG test, the MEA measurement on spontaneously active heart cells shows that no appreciable QT change is caused.

It follows from this that measurements on only one channel (such as HERG) do not correctly reflect the complex inter-relationships and may lead to false-positive results, whereas this is not the case in MEA measurements (via FPdur).

As a control, Fig. 4 plots the QT changes for the above-discussed quinidine and for E4031 and sotalol, each of which is known to lead to a QT prolongation.

For quinidine, the prolongation of the QT interval in the ECG has been described by a great many authors, and it is recognized by the FDA. Likewise, the FDA confirms an influence of quinidine on the occurrence of tachycardia/torsades de pointes leading to ventricular fibrillation. The QT prolongation was already demonstrated in the 70s. More recent works show an inhibitory effect of quinidine on heterologously expressed HERG channels, as a molecular mediator of QT prolongation.

QT prolongations are already shown *in vitro* in the MEA system at therapeutic concentrations in the range of 2 – 7 μM.

Sotalol, (N-[4-[1-hydroxy-2-(isopropylamino)ethyl]phenyl] methanesulfonamidehydrochloride, is also used as an antiarrhythmic agent. QT prolongation and triggering of torsades de

pointes have been described in many instances and accepted as side effects. The risk of torsades de pointes is much higher in female patients than in male patients. In the measurements carried out here, a clear prolongation of the action potential was shown (about double). For the known effects of sotalol, reference is made, for example, to: Farkas A., Lepran I., Papp J.G.: Proarrhythmic effects of intravenous quinidine, amiodarone, D-sotalol, and almokalant in the anesthetized rabbit model of torsade de pointes; J. Cardiovasc. Pharmacol. 2002 Feb; 39(2):287-297.

E4031, {4'-[[1-[2-(6-methyl-2-pyridinyl)ethyl-4-piperidinyl]carbonyl]methanesulfonamide, 2HCl}, is not a medication, but a highly selective inhibitor of I(Kr) current. This current is responsible for the repolarization of the ventricular action potential. In terms of molecular biology, the channel through which most of the I(Kr) current flows is referred to as HERG (human ether-a-gogo-related gene). Since HERG channels are often used in heterologous expression systems as *in vitro* assay for a potential QT prolongation, this substance is of particular importance as a reference.

The sensitivity of the MEA system is also clear from the fact that even submicromolecular concentrations of E4031 lead to inhibition of the I(Kr) current, which is expressed in a prolongation of the ventricular action potential of over 90%. At higher concentations, the cells no longer have any spontaneous contractions. For the effect of E4031, see for example Webster R., Allan G., Anto-Awuakye K., Harrison A., Kidd T., Leishman D., Walker D.: Pharmacokinetic/pharmacodynamic assessment of the effects of E4031, cisapride, terfenadine and terodiline on

monophasic action potential duration in dog, Xenobiotica. 2001 Aug-Sep;31(8-9):633-650.

In so far, the inventors could show the prolongating effect of quinidine, amiodarone (antiarrhythmic agent), terfenadin (antihistaminic agent), astemizol (antihistamic agent), E-4031, cisapride (prokinetic agent), sotalol and fexofenafine (antihistaminic agent) (the latter only at very high concentrations), respectively, on the QT interval. No QT prolongation was found for verapamil (HERG blocker) and ivabradine (IKF blocker, regulates pace maker current).

Further, two substances with so far unknown effect on QT interval have been tested, whereby rilmakalim (IKATP opener) reduced the frequence but did not prolong the field potential, but H1098 had a remarkably prolonged field potential.

Claims

1. Method for determining the influence of a test substance on the heart activity of a vertebrate, comprising the following steps:

a) preparation of a culture of spontaneously active heart cells of the vertebrate,

b) extracellular measurement or recording of electrophysiological data of the heart cells from step a),

c) addition of the test substance to the culture from step a),

d) repetition of the measurement from step b), and

e) comparison of data from step b) and step d).

2. Method according to claim 1, characterized in that the influence of the test substance on the duration of ventricular depolarization and repolarization (QT interval) is determined.

3. Method according to claim 1 or 2, characterized in that the test substances are chosen from the group: approved medicaments, and medicaments for approval, from human and

veterinary medicine, environmental factors, cosmetic products, environmental pollutants.

4. Method according to anyone of claims 1 to 3, characterized in that, in step a), the heart cells cultured are individual heart cells, aggregates of heart cells, or tissue composites of heart cells.

5. Method according to anyone of claims 1 to 4, characterized in that, in step a), the heart cells used are native heart cells, for example from biopsies or clinical material, heart cells derived from stem cells, or heart cell lines.

6. Method according to anyone of claims 1 to 5, characterized in that it is performed in the context of a diagnostic procedure or in the run-up to a therapeutic procedure.

7. Method according to anyone of claims 1 to 6, characterized in that it is performed in the context of an individual choice or production of a medicament for an individual patient.

8. Method according to anyone of claims 1 to 7, characterized in that a MEA is used.

9. Method according to anyone of claims 1 to 7, characterized in that a plurality of separate cultures of heart cells are cultured on a support plate and measured.

10. Method according to claim 9, characterized in that, in the support plate, a plurality of wells or microcuvettes are provided, in each of which a culture is held.

11. Method according to claim 10, characterized in that, in each well or in each microcuvette, at least one measurement electrode is provided for recording of the field potential.

12. Method according to anyone of claims 1 to 11, characterized in that the vertebrate is a mammal, in particular a livestock animal, a domestic animal, or a human.

13. Method according to anyone of claims 1 to 12, characterized in that, in steps b) and d), data are derived concerning the course or trace of the field potential (FP).

14. Method according to claim 13, characterized in that at least one parameter characterizing the FP course is calculated from the course or trace of the field potential.

15. Method according to claim 14, characterized in that FPdur and/or FPrise are calculated and compared.

16. Method according to anyone of claims 1 to 15, characterized in that, in step e), the influence of the test substance on heart activity, preferably on the QT interval, is determined.

17. Use of a device for extracellular recording of electrophysiological data, for example a MEA or a support plate

with microcuvettes or wells in each of which there is at least one measurement electrode, for determining the influence of a test substance on the heart activity of a vertebrate.

18. Use according to claim 17 in a method according to anyone of claims 1 to 16.

19. A method for treating a human individual or an animal, comprising the steps of

a) selecting a test substance suitable for said treatment,

b) providing heart cells of said human individual or animal, respectively,

c) determining the influence of said test substance on the heart activity of said human individual or animal, respectively, by the method of anyone of claims 1 – 16,

d) preparing a pharmaceutical composition containing said test substance in case said influence on the heart activity is admissible.

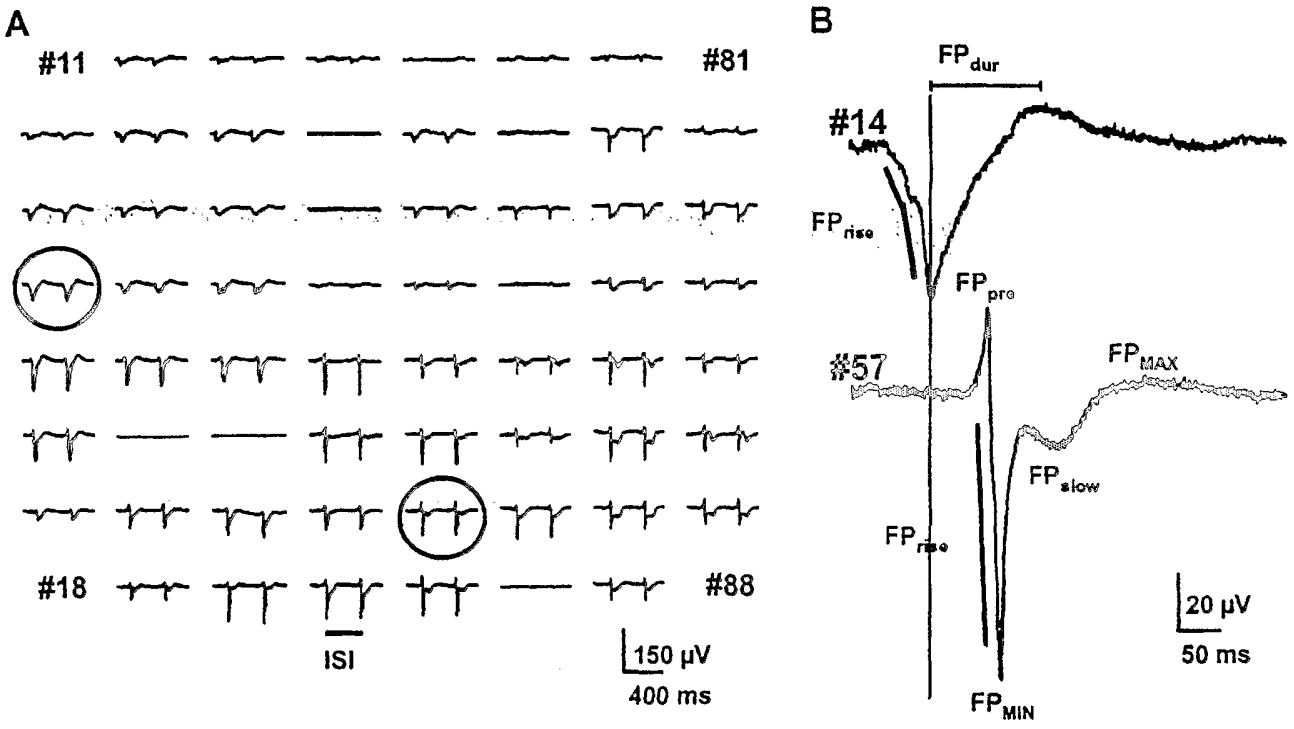
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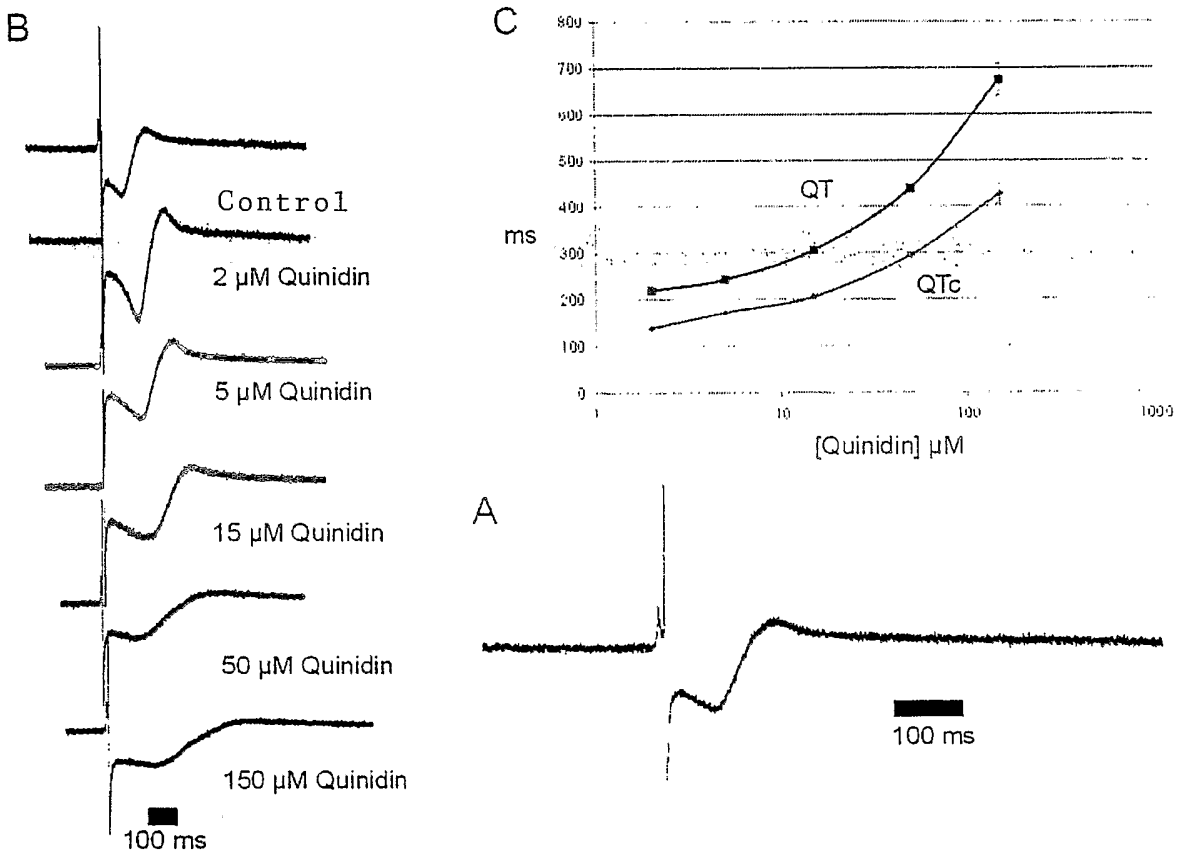
Fig. 1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150 µM Quinidin
100 ms
C
ms
QT
QTc
[Quinidin] µM
A
100 ms

Fig. 2

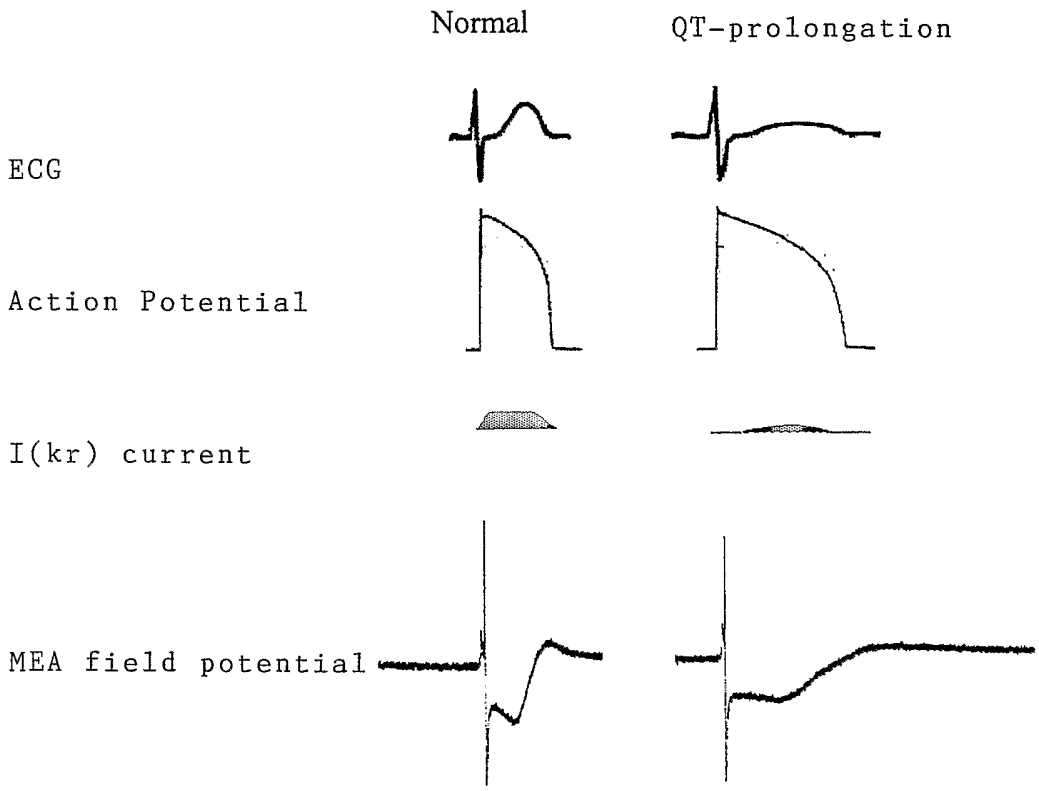
Normal
QT-prolongation
ECG
Action Potential
I(kr) current
MEA field potential

Fig. 3

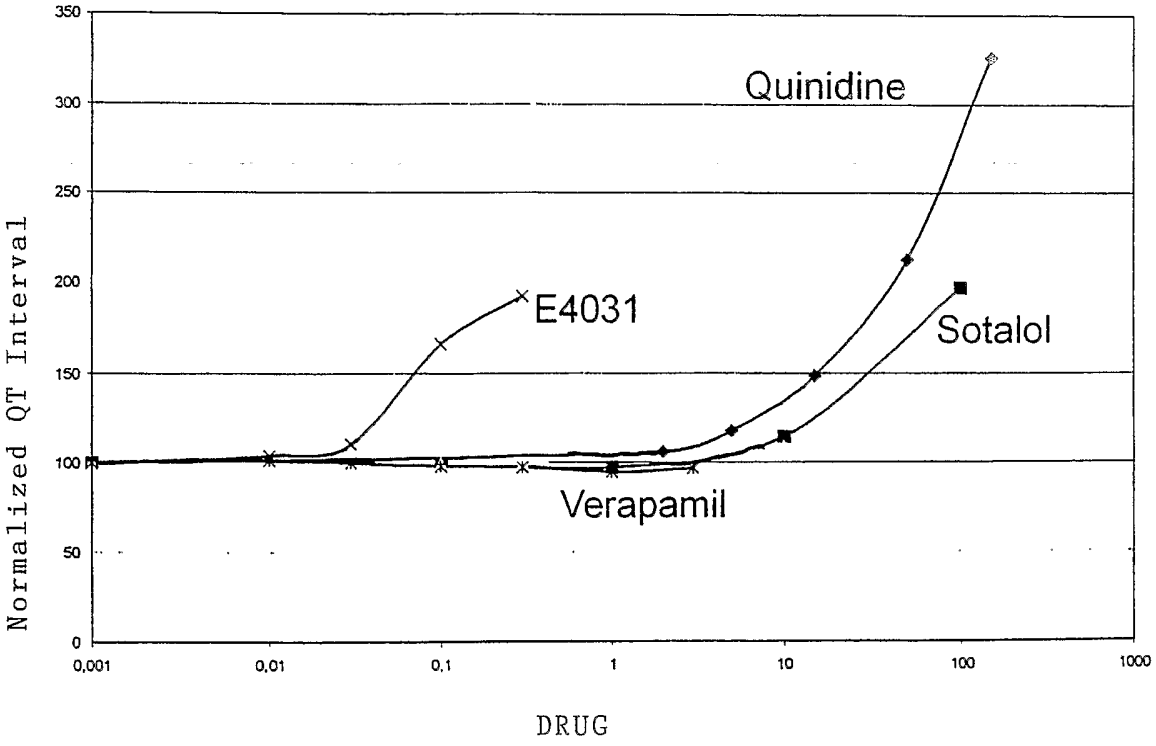
Normalized QT Interval
350
300
250
200
150
100
50
0
0,001
0,01
0,1
1
10
100
1000
DRUG
Quinidine
E4031
Sotalol
Verapamil

Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/000717

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06 G01N33/50 G01N33/487 C12M1/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IGELMUND PETER ET AL: "Action potential propagation failures in long-term recordings from embryonic stem cell-derived cardiomyocytes in tissue culture" PFLUEGERS ARCHIV EUROPEAN JOURNAL OF PHYSIOLOGY, vol. 437, no. 5, April 1999 (1999-04), pages 669-679, XP002274096 ISSN: 0031-6768 page 670, left-hand column, paragraph 2 -page 671, left-hand column, paragraph 1; figure 1 --- -/--	1,3-6, 8-14, 16-18

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2004	24/06/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wilhelm, J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/000717

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DENYER M C T ET AL: "PRELIMINARY STUDY ON THE SUITABILITY OF A PHARMACOLOGICAL BIO-ASSAYBASED ON CARDIAC MYOCYTES CULTURED OVER MICROFABRICATED MICROELECTRODE ARRAYS" MEDICAL AND BIOLOGICAL ENGINEERING AND COMPUTING, PETER PEREGRINUS LTD. STEVENAGE, GB, vol. 36, no. 5, 1 September 1998 (1998-09-01), pages 638-644, XP000777560 ISSN: 0140-0118 paragraph '2.2.! - paragraph '004.! ---	1,3-6,8, 12,13, 16-18
X	ISRAEL D A ET AL: "AN ARRAY OF MICROELECTRODES TO STIMULATE AND RECORD FROM CARDIAC CELLS IN CULTURE" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 247, no. 4 PART 2, 1984, pages H669-H674, XP008028842 ISSN: 0002-9513 page H670, right-hand column, paragraph 2 - paragraph 4 page H674, right-hand column ---	1,3-6,8, 12,13, 16-18
Y	MEIRY GIDEON ET AL: "Evolution of action potential propagation and repolarization in cultured neonatal rat ventricular myocytes" JOURNAL OF CARDIOVASCULAR ELECTROPHYSIOLOGY, vol. 12, no. 11, November 2001 (2001-11), pages 1269-1277, XP008028795 ISSN: 1045-3873 page 1269, left-hand column -page 1270, left-hand column; figure 5 ---	1-6,8, 12-18
Y	DUFFY P A ET AL: "Preclinical studies of the effects of quetiapine on electrocardiographic measurements" SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 27, no. 2, 2001, page 1766 XP002274097 31st Annual Meeting of the Society for Neuroscience;San Diego, California, USA; November 10-15, 2001 ISSN: 0190-5295 abstract -----	1-6,8, 12-18

INTERNATIONAL SEARCH REPORT

national application No.
PCT/EP2004/000717

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 19
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.